Growing up is losing some illusions, in order to acquire others.

- Virginia Woolf

Members of the Examination Committee:

Prof. Dr. ir. Korneel Rabaey (Chairman)
Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University

Dr. Andrew Hunt
Green Chemistry Centre of Excellence, Department of Chemistry, University of York, United Kingdom

Prof. Dr. ir. Bert Sels
Centre for Surface Chemistry and Catalysis, Faculty of Bioscience Engineering, KU Leuven

Prof. Dr. ir. Guy Marin
Department of Chemical Engineering and Technical Chemistry, Faculty of Engineering and Architecture, Ghent University

Prof. Dr. ir. Marjan De Mey
Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University

Prof. Dr. ir. Sven Mangelinckx
Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University

Prof. Dr. ir. Christian Stevens (Promotor)
Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University

Prof. Dr. ir. Kevin Van Geem (Co-promotor)
Department of Chemical Engineering and Technical Chemistry, Faculty of Engineering and Architecture, Ghent University

This work is supported by the Long Term Structural Methusalem Funding by the Flemish Government (grant number BOF09/01M00409).

Promotor: Prof. Dr. ir. Christian Stevens
Department of Sustainable Organic Chemistry and Technology
Faculty of Bioscience Engineering
Ghent University

Co-promotor: Prof. Dr. ir. Kevin Van Geem
Department of Chemical Engineering and Technical Chemistry
Faculty of Engineering and Architecture
Ghent University

Dean: Prof. Dr. ir. Marc Van Meirvenne

Rector: Prof. Dr. Anne De Paepe

ir. Elisabeth Delbeke

Innovative sophorolipid analogues with tailor-made physico-chemical and biological properties

Thesis submitted in fulfilment of the requirement for the degree of
doctor (PhD) in Applied Biological Sciences: Chemistry and Bioprocess Technology

Dutch translation of the title:

Innovatieve sophorolipide analoga met *tailor-made* fysico-chemische en biologische eigenschappen

Please cite as:

E. Delbeke, 'Innovative sophorolipid analogues with tailor-made physico-chemical and biological properties', PhD Dissertation, Ghent University, 2016

Cover illustration:

Coriandrum sativum seeds (Societa Cofica Pty Ltd), *Saccharum officinarum* (Alive feel), Vacuum concentrating (Chris Young), *Brassica napus* flowers (BioSpectrum Bureau), *Starmerella bombicola* (Karen Saerens).

ISBN number: 978-90-5989-884-4

Ghent, May 2016

The author,

ir. Elisabeth Delbeke

The promotors,

Prof. Dr. ir. Chris Stevens

Prof. Dr. ir. Kevin Van Geem

Woord vooraf

September 2012, met de start van dit doctoraatsproject ging er voor mij een nieuw hoofdstuk van start dat nu, na heel wat ups en downs, tot een einde is gekomen. Het resultaat van deze jaren onderzoek heeft u voor zich liggen. Deze thesis had niet gerealiseerd kunnen worden zonder de hulp van een aantal mensen, die ik dan ook graag wil bedanken.

In de eerste plaats wil ik mijn promotor, Prof. Chris Stevens, bedanken om mij de mogelijkheid te bieden onder zijn promotorschap deze doctoraatsthesis uit te werken. U heeft mij steeds de vrijheid gegeven om mijn eigen weg te zoeken in dit onderzoek met de nodige goede raad op de juiste momenten. In het kader van dit project heeft u mij de kans geboden om deel te nemen aan de visioning workshop rond hernieuwbare grondstoffen in het Biobase Europe Training Centre in Terneuzen en de RRB-conferentie in Valladolid, beide een unieke ervaring. Daarnaast heeft u mij ook met verschillende mensen in contact gebracht die onontbeerlijk waren voor dit onderzoek.

Ook mijn co-promotor, Prof. Kevin Van Geem, wil ik bedanken voor zijn begeleiding bij deze doctoraatsthesis. Met veel enthousiasme heeft u zich vanaf de eerste dag voor dit project ingezet. Al stond het onderwerp van deze thesis u wat verder af dan uw eigen expertise, toch heeft u steeds de tijd genomen om alles uitgebreid door te nemen en te bespreken. Ik waardeer uw goede raad en alle kansen die u mij geboden heeft, zoals het presenteren van dit onderzoek op de ACS meeting in San Francisco. Hierbij aansluitend wil ik dan ook Prof. Guy Marin bedanken die mij het vertrouwen heeft gegeven om met de financiering van dit project op het Methusalem budget deze kansen mogelijk te maken.

I would also like to thank Prof. Korneel Rabaey, Dr. Andrew Hunt, Prof. Bert Sels, Prof. Guy Marin, Prof. Marjan De Mey and Prof. Sven Mangelinckx for being part of the examination committee. I highly valuate your thorough reading of this manuscript and your comments which allowed me to improve the quality of this thesis.

Voor het uitwerken van verschillende aspecten van deze doctoraatsthesis heb ik beroep kunnen doen op de expertise van verschillende mensen. Ik wil graag Inge, Sophie en Stijn van de InBio onderzoeksgroep bedanken voor het aanleveren van het startproduct voor dit project en voor hun hulp bij alles wat met fermentatie te maken had. Quenten en Prof. Paul Van Der Meeren van de vakgroep Toegepaste Analytische en Fysische Chemie wil ik bedanken voor hun hulp bij het bepalen van de oppervlakte spanningen en het interpreteren van deze resultaten. Dr. Niki Baccile wil ik bedanken voor het uitvoeren van de SAXS-analyses. Prof. Tom Coenye wil ik bedanken voor het

bepalen van de antimicrobiële activiteit van de derivaten. Prof. Paul-Alain Jaffrès en zijn medewerkers wil ik bedanken voor het analyseren van de transfectie eigenschappen.

Daarnaast wil ik ook graag alle collega's bedanken naast wie ik de afgelopen jaren in het labo heb gewerkt. Nicola en Ewout, als jaargenoten zijn we samen aan deze uitdaging begonnen. Eerst samen in het labo voor onze masterproef en nadien ook voor ons doctoraat, dat schept toch wel een band. Stein, Arno, Jeroen en Benz, het was een plezier om met jullie een bureau te delen. Ook al is het bureau op het gelijkvloers niet al te populair bij onze collega's, wij hebben er toch maar onze vaste stek van gemaakt. Stein en Arno, samen vormden we dan ook nog eens de basis van de renewables groep en de begeleiding van de polymeren practica. Bart, jij stond altijd klaar met goede raad als ik met mijn vragen bij jou kwam. Heel erg bedankt daarvoor. Ans, Pieter en Els, jullie staan dag in dag uit klaar om ons te helpen met al onze praktische problemen. Jullie zorgen ervoor dat alles goed blijft lopen in het labo en zijn voor ons dan ook onmisbaar. Ook alle andere collega's, ik ga niet iedereen bij naam noemen, wil ik bedanken voor de toffe sfeer en leuke momenten gedurende de afgelopen jaren. Mijn studenten die ik de afgelopen jaren begeleid heb (Jelle, Jan-Klaas, Evelien, Etienne en Jonas) wil ik bedanken voor hun inzet en hun enthousiasme voor groene chemie.

Mijn vrienden en familie wil ik bedanken voor hun steun de afgelopen jaren en om er altijd voor mij te zijn. Manon, mijn reisgenootje, samen hebben we al heel wat meegemaakt. Jammer genoeg ben je het afgelopen jaar naar Engeland verhuisd en zie ik je niet meer zoveel als ik wel zou willen, toch weet ik dat ik steeds bij jou terecht kan. Lies en Elien, onze vriendschap gaat al meer dan 20 jaar terug, bedankt om er al die jaren voor mij te zijn. Vincent, Sofie, Nicolas en Ines, bedankt voor alle mooie momenten die we de afgelopen jaren gedeeld hebben, voor jullie steun en vriendschap. Piet, Tine en Sebastiaan, mijn ouders en broer, bedankt om er altijd voor mij te zijn en mij in alles wat ik doe te steunen.

En *last but not least* wil ik Christophe bedanken, ik kan mij de afgelopen jaren niet indenken zonder jou aan mijn zijde. Jij begreep als geen ander de vreugde en frustraties die gepaard gaan met het uitwerken van een doctoraat. Bedankt voor jouw steun, liefde en alle onvergetelijke momenten samen.

Elisabeth Delbeke

Mei 2016

Table of contents

List of abbreviations

A	Surface coverage area per surfactant
Ac	Acetyl
ACN	Acetonitrile
ADP/ATP	Adenosine di/triphosphate
AE	Atom economy
AIBN	Azobisisobutyronitrile
ATR	Attenuated Total Reflectance
aq.	Aqueous
Bn	Benzyl
Boc	*t*-butoxycarbonyl
BPO	Benzoyl peroxide
Bt	Benzotriazole
Bu	Butyl
Bz	Benzoyl
CAL-B	*Candida antarctica* lipase
cat.	Catalyst
CE	Carbon efficiency
CFTR	Cystic fibrosis transmembrane conductance regulator
CFU	Colony-forming units
CoA	Coenzyme A
COSY	Correlation spectroscopy
CTPS	Control tissue culture polystyrene
CMC	Critical micelle concentration
CR	Charge ratio
CSL	Corn steep liquor
CV	Column volume
d	Day(s)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	Dichloroethane
DCC	*N*,*N*'-dicyclohexylcarbodi-imide
DDAB	Didodecyldimethylammonium bromide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	Diisopropyl azodicarboxylate
DIC	*N*,*N*'-diisopropylcarbodiimide
DIEA/DIPEA	*N*,*N*-diisopropylethylamine
DLS	Dynamic light scattering
DMAP	4-dimethylaminopyridine
DMC	Dimethyl carbonate
DMSO	Dimethylsulfoxide
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DOPE	1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine
DPPA	Diphenylphosphoryl azide
DSC	Differential scanning calorimetry
DSP	Downstream processing
EC	Effective concentration
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

ELSD	Evaporative light-scattering detection
eq	Equivalents
Et	Ethyl
Fmoc	Fluorenylmethyloxycarbonylchloride
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GG	Gellan gum
h	Hour(s)
HIV	Human immunodeficiency virus
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation
IC	Inhibitory concentration
IgE	Immunoglobuline E
*i*Pr	Isopropyl
LA	Linolenic acid
LC-MS	Liquid chromatography – Mass spectroscopy
LDH	Lactate dehydrogenase
LFM	Lipofectamine
MAEE	Mono-acetylated ethyl ester sophorolipid
MBC	Minimum bactericidal concentration
*m*CPBA	3-chloroperoxybenzoic acid
Me	Methyl
MIC	Minimum inhibitory concentration
MLD	Minimum lethal doses
MML	*Mucor miehei* lipase
mRNA	Messenger RNA
MRSA	meticillin-resistant *Staphylococcus aureus*
MS	Molecular sieves or mass spectroscopy
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUFA	Monounsaturated fatty acid
NAD^+/NADH	Nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
NHS	*N*-hydroxysuccinimide
NMMO	*N*-methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
NOEC	No-observed-effect concentration
Ns	Nosyl / 4-nitrobenzene-1-sulfonyl
OA	Oleic acid
OD	Optical density
oe *sble*	*Starmerella bombicola* lactone esterase overexpression strain
o/w	Oil/water
PA	Petroselinic acid
Paba	*p*-aminobenzoic acid
PBS	Phosphate-buffered saline
PDC	Pyridinium dichromate
pDNA	Plasmid DNA
PMB	*p*-methoxybenzyl
PPG	Polypropylene glycol
PPL	Porcine pancreatic lipase

PPTS	Pyridinium *p*-toluenesulfonate
PSL	*Pseudomonas* sp. lipase
PUFA	Polyunsaturated fatty acid
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
quant.	Quantitative yield
QD	Quantum dot
RME	Reaction mass efficiency
RNA	Ribonucleic acid
ROMP	Ring-opening metathesis polymerization
rt	Room temperature
SAXS	Small-angle X-ray scattering
SF	Stoichiometric factor
SFA	Saturated fatty acid
shRNA	Small hairpin RNA
siRNA	Small interfering RNA
SL	Sophorolipid
TBDPS	*t*-butyldiphenylsilyl
TBME	*t*-butyl methyl ether
TE	Transfection efficacies
TEG	Triethylene glycol
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMDSC	Temperature modelated DSC
TMSH	Trimethylsulfonium hydroxide
TMSOTf	Trimethylsilyltriflate
TPA	12-*O*-tetradecanoylphorbol-13-acetate
UDP	Uridine diphosphate
UV	Ultraviolet
v/v	Volume/volume percentage
WHO	World Health Organization
w/o	Water/oil
w/v	Weight/volume percentage
XRD	X-ray diffraction
Δ	Reflux
γ_{min}	Minimum surface tension
Γ_m	Maximum surface adsorption density
σ	Interfacial tension

Glossary

Amphiphile	Chemical compound possessing both hydrophilic and hydrophobic properties.
Biosurfactant	Surfactant produced from renewable resources
Bolaamphiphile	Amphiphilic compounds which contain two hydrophilic parts linked by a hydrophobic linker.
Compaction (of pDNA)	Formation of a complex between pDNA and a liposomal formulation.
Charge ratio (CR)	Term used in transfection experiments to indicate the number of positive charges provided by the cationic lipid derivative divided by the number of positive charges carried by the pDNA.
Critical micelle concentration (CMC)	Lowest concentration of surfactants for which micelles are formed.
Fermentation	Bioconversion where yeast or bacteria convert certain substrates (mostly sugars) into other compounds such as for example ethanol, lactic acid or sophorolipids.
Guinier analysis	Method to calculate the particle size of micelles in SAXS analysis.
In vitro	Studies performed with micro-organisms, cells or biological molecules outside their biological context.
In vivo	Studies performed with whole living organisms.
Liposomal formulation	Aggregation of amphiphiles into spherical vesicles.
Lipoplex	Complex between pDNA and a liposomal formulation.
Minimum bactericidal concentration (MBC)	The lowest concentration of test compound in antimicrobial evaluation at which no more viability of the test organism can be observed; indication of microbial death.
Minimum inhibitory concentration (MIC)	The lowest concentration of test compound in antimicrobial evaluation for which a lack of visible bacterial growth is observed; indication of microbial growth inhibition.
SAXS analysis	Small-angle scattering technique where the scattering of X-rays by a sample of self-assembled aggregates is recorded at very low angles to obtain information on the shape and size of the aggregates.
Surfactant	Chemical compound possessing both hydrophilic and hydrophobic properties that can lower the surface or interfacial tension.
Supramolecular aggregate	Well defined complex of molecules held together by noncovalent bonds.
Transfection	The process of introducing nucleic acids (DNA) into cells.
Transmission electron microscopy (TEM)	Microscopic technique to visualize small objects (nm scale) by transmitting a beam of electrons through a very thin sample.
Wilhelmy plate method	Measurement of the surface tension of a solution with a thin, rectangular plate which has optimal wetting properties.

1. Introduction and goals

In the last decades, the transition towards a bio-based economy has been initiated. This transition focusses on the use of renewable resources and sustainable technologies as replacements for the classical, fossil based chemical industry.[1-4] In view of the agreements made at the COP21 conference on climate change in Paris (November 2015) which resulted in the first-ever universal, legally binding climate deal, this transition is becoming more urgent than before.

For the further development of a bio-based economy, the implementation of biorefineries will play an important role in the future.[5-8] Biorefineries focus on the complete utilization and conversion of biomass into a variety of valuable products. The production of transportation fuels is seen as the driving force for the development of biorefinery units and the accessibility of new chemical platform molecules. Therefore, biorefineries mostly combine the production of high-volume transportation fuels with the production of low-volume chemicals with a higher added-value. For example, an increased production of biodiesel will result in an increased supply of the by-product glycerol. To date, the conversion of biomass and its implementation in a biorefinery still faces a lot of challenges. The biggest bottleneck is the economic viability of the generated products which can often hardly compete with fossil based alternatives, certainly with the current low oil prices of 50 $/barrel. Other problems which should be dealt with are related to transport and seasonal variability of biomass.[1]

At present, only 8% of all the chemicals produced in Europe are based on renewable resources.[9] Although the application of renewable resources is not a new concept, this mostly comprises the use of simple resources or unpurified products for the production of low added-value products such as plastics, detergents, surfactants, paper and textile. The application of renewable resources in high added-value products, often having a complex structure, faces much more challenges since multiple reaction steps are needed to obtain the desired products. When renewable resources are used for the production of high-added value products, they are mostly broken down into low molecular weight base-chemicals which are subsequently used as building blocks for the synthesis of more complex compounds. Due to the high price of these renewable base-chemicals, they can hardly compete with fossil based products for high added-value applications. However, these high added-value applications offer the best perspective for the succesfull implementation of biorefineries.

When renewable resources with a complex structure would directly be used as building blocks for chemical derivatization, the number of reaction steps and the associated production cost can be drastically reduced. This approach makes optimal use of the high complexity inherent to these compounds and the high oxidation state they already possess. The most critical points to develop an economical viable synthesis pathway are the purity and quantity of the renewable feedstock.

In this respect, sophorolipid biosurfactants can be regarded as excellent renewable resources for chemical derivatization. They are glycolipid biosurfactants, produced by different yeast species *via* fermentation of renewable resources.[10-11] *Starmerella bombicola* is the preferred production organism, for which a production up to 400 g/L was reported.[10] Sophorolipids consist of sophorose as hydrophilic head and a fatty acid (mostly oleic acid) as lipid tail, which gives them an amphiphilic character. Sophorolipids lower the surface tension of water from 72.8 to 30-40 mN/m and have a critical micelle concentration of 40-100 mg/L.[10] Their emulsifying properties can be applied for the recovery of oil and hydrocarbons and for the decontamination of soil and water.[12-17] Microbial production results in the formation of different sophorolipid derivatives with diacetylated C18:1 sophorolipid lactone **1** and C18:1 sophorolipid acid **2** being the major fermentation compounds (Figure 1). Natural sophorolipids possess interesting biological activities, such as anti-cancer, antimicrobial, dermatological, immunoregulatory, spermicidal and antiviral activities.[18-19] Besides, they possess self-assembly properties which results in the formation of nanostructures with supramolecular chirality.[20-21] Selective production of specific sophorolipid lactone or acid derivatives can be obtained with genetically modified *S. bombicola* strains.[22] When feeding pure hydrophobic substrates to these modified strains, production of one single sophorolipid derivative can be obtained.[23]

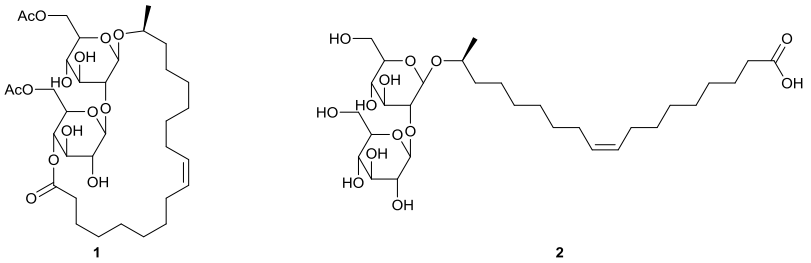


Figure 1. Diacetylated sophorolipid lactone 1 and sophorolipid acid 2

The incorporation of sophorolipid production in current biorefineries is feasible since they can be produced from waste streams such as biodiesel by-product streams.[11] However, fermentations using these cheap substrates will result in lower yields and a more heterogeneous product compared to fermentations using glucose and oleic acid. Therefore, when sophorolipids are considered as building blocks for chemical derivatization, the production facility should have as main focus the production of sophorolipids and not transportation fuels.

Sophorolipids are considered as one of the most promising classes of biosurfactants, mainly due to their production as a homogenous product in high quantity.[10, 24] In contrast to the first generation biosurfactants, such as the alkylpolyglucosides (APGs) which are produced chemically from renewable resources, sophorolipids belong to the second generation biosurfactants which are

produced biotechnologically by micro-organisms. This offers the advantage that they contain a rare hydrophilic head (sophorose) in combination with a specific chirality in the lipid tail and at the anomeric positions, both features which are not easily accessible via chemical synthesis. The second generation biosurfactants include amongst others phospholipids, surfactin, emulsan and other glycolipids such as rhamnolipids, trehalose lipids, cellobiose lipids and mannosylerythritol lipids.

Interest in sophorolipids exponentially increased over the last 20 years, reaching up to 600 citations in 2013, 2014 and 2015 (Figure 2). To date, natural sophorolipids are commercialized by different companies world-wide. The Japanese company Saraya and the Belgian company Ecover apply sophorolipids in their laundry, dishwashing and cleaning products while the former French company Soliance, now part of the Swiss company Givaudan, and the Korean company MG Intobio offer respectively the products Sopholiance S and Sopholine for cosmetic applications.[22] Other companies working on the industrial production of sophorolipids are Evonik, DSM and Croda.[22, 25]

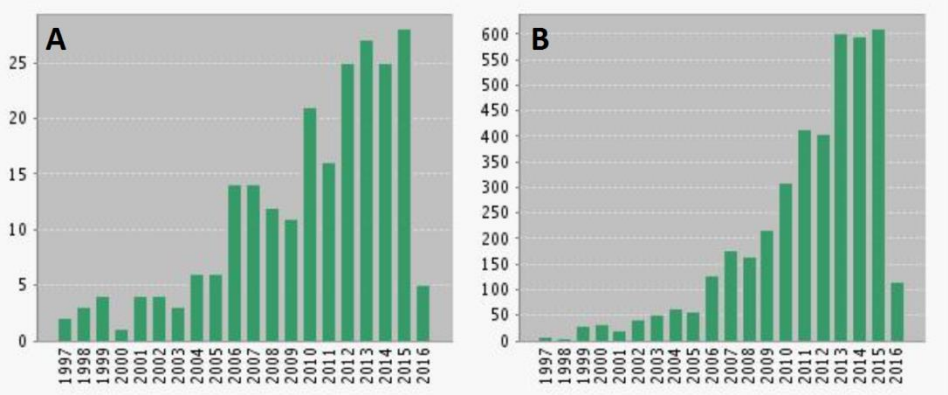


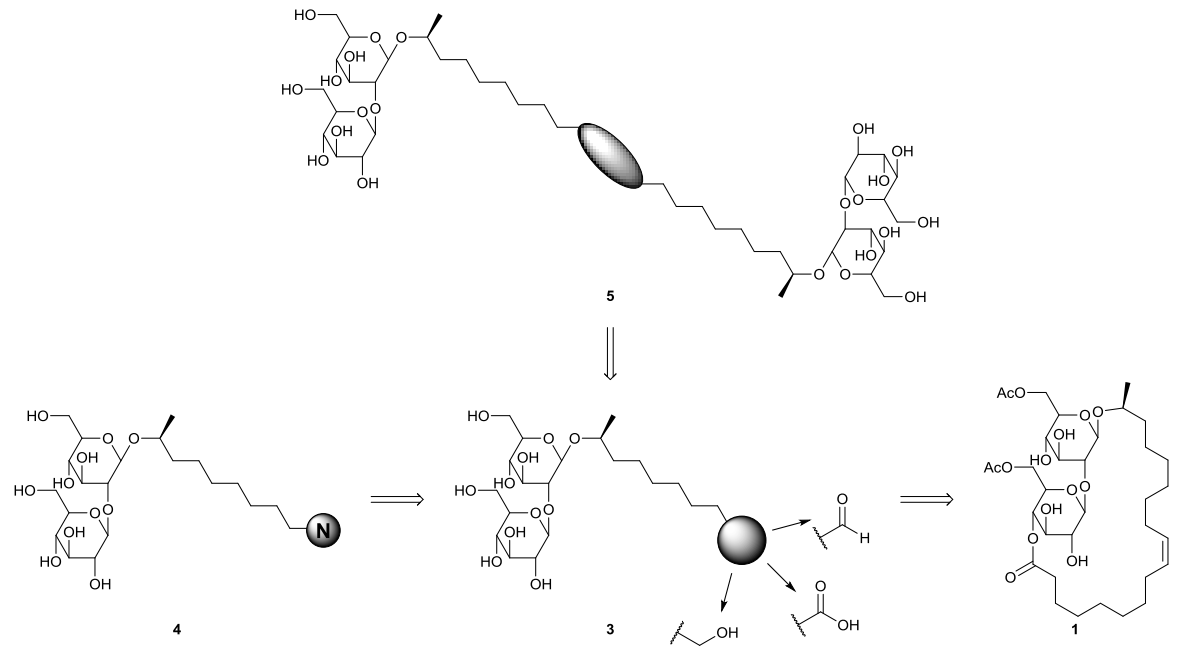
Figure 2. Number of publications (A) and citations (B) per year on sophorolipids. These data was retrieved from the Web of Science database with the term "Sophorolipid" as key-word.

Unfortunately, several disadvantages restrict the application potential of the natural sophorolipids. First of all, their production cost is estimated at 2 to 5 €/kg although an up-to-date production price is not communicated by the companies.[10] Secondly, the sophorolipid microbial production pathway limits the fatty acid incorporation to C16 or C18 hydrophobic substrates. This selectivity originates from the specificity of the hydroxylation step of the hydrophobic substrates and can be circumvented by using already hydroxylated substrates or stearic acid-like substrates for the production of shorter-chain derivatives.[26] For example, the microbial production of C12 sophorolipid compounds was successfully accomplished using 12-hydroxydodecanoic acid, 1-dodecanol or 1,12-dodecanediol.[26-27] Secondary alcohols can be used as well, but only the *(S)*-enantiomers will be incorporated in the sophorolipid product. This was confirmed by fermentations using ricinoleic acid having an *(R)*-hydroxyl function, which was not included in the sophorolipid structure.[11]

Chemical modification offers the opportunity to extend the limited set of microbial derivatives to other high-added value sectors, in particular the pharmaceutical sector. Compared to the other second generation biosurfactants, sophorolipids offer several advantages as building blocks for chemical modification towards high-added value products. Sophorolipids are produced by a non-pathogenic yeast, in contrast to the rhamnolipids which are produced by the pathogen *Pseudomonas aeruginosa*. Moreover, sophorolipids can be produced in high quantity, in contrast to most of the other glycolipids, and the production of one homogenous compound can be accomplished, in contrast to the phospholipids.

At present, chemical and enzymatic modifications of sophorolipids have been mostly limited to the sugar head or the lipid tail.[19] Cleavage of the double bond, however, has only been described for the synthesis of short-chained sophorolipid acids or in ring-opening cross-metathesis reactions.[28-29] It was never included in a synthetic pathway for the production of a functionalized building block towards modified derivatives.

The goal of this research project is the synthesis of a wide range of innovative sophorolipid derivatives with optimized surface-active properties and biological activities by combining the fermentation technology with chemical derivatization (Scheme 1). In view of the high production cost of the microbialy produced sophorolipids, application of the modified derivatives as detergents will not be feasible. Therefore, it is the aim for this project to synthesize innovative derivatives with a high added-value which can be applied in the pharmaceutical sector where they can be economically competitive.



Scheme 1. Retrosynthetic scheme for the synthesis of innovative sophorolipid derivatives

A first goal of this research project is the synthesis of functionalized sophorolipid building blocks **3** with focus on the synthesis of a sophorolipid aldehyde intermediate. Therefore, an ozonolysis reaction will be performed to transform the double bond into a reactive site. This modification will reduce the chain length of the sophorolipid derivatives which will increase their hydrophilic character.

A second goal of this research project is the transformation of the sophorolipid building blocks **3** into a broad library of innovative sophorolipid derivatives. On the one hand, a nitrogen functionality will be introduced. This offers the opportunity to synthesize a whole range of nitrogen containing sophorolipid derivatives **4** such as sophorolipid amines, sophorolipid quaternary ammonium salts and sophorolipid amine oxides. All these derivatives can possess cationic surfactant properties, depending on the pH of the solution which can have a great influence on their solubility and biological activity. On the other hand, the sophorolipid building blocks **3** can be used for the synthesis of a new class of sophorolipid bolaamphiphiles **5**. These compounds contain two hydrophilic parts linked by a hydrophobic linker and they can be used for the formation of stable liposomes and vesicles. Therefore, they offer perspectives for targeted drug delivery.

For each class of derivatives, a lot of attention will be payed to the evaluation of their surface-active properties and biological activities. All derivatives will be evaluated on their antimicrobial activities against both Gram-positive and Gram-negative bacteria. Their self-assembly properties will be assessed *via* the measurement of their critical micelle concentration (CMC) and *via* small-angle X-ray scattering (SAXS) analysis. The derivatives which possess a permanent positive charge will be evaluated on their suitability as gene delivery vectors.

2. State of the art: Chemical and enzymatic modification of sophorolipids

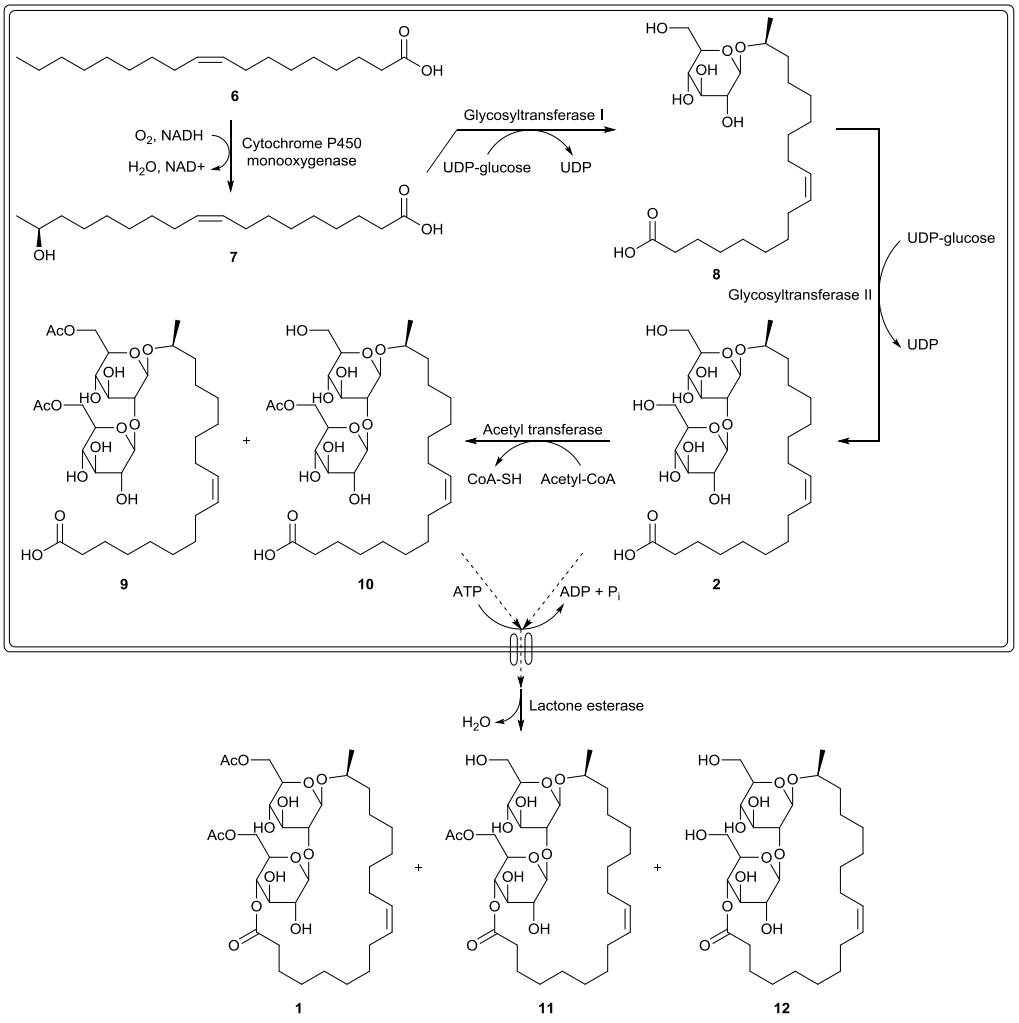
2.1. Production of natural sophorolipids

Sophorolipids are produced by a selected number of yeast species.[10-11, 30] They were first described by Gorin *et al*. in the early 1960s as an extracellular glycolipid produced by *Candida apicola*, formerly known as *Torulopsis apicola*. Other producing strains comprise *Rhodotorula bogoriensis* (formerly known as *Candida bogoriensis*), *Starmerella bombicola* (formerly known as *Torulopsis bombicola* and *Candida bombicola*) and *Wickerhamiella domericqiae*. *Starmerella bombicola* is the best known and preferred producing strain with a production yield of more than 400 g/L. The sophorolipid biosynthesis starts with glucose as the hydrophilic carbon source and fatty acids, fatty acid methyl esters, triglycerides or alkanes as the hydrophobic carbon source (Scheme 2). All hydrophobic carbon sources are converted into fatty acids, which contain in general 16 or 18 carbon atoms with one or more double bonds. When no hydrophobic carbon source is present in the fermentation medium, fatty acids can be formed *de novo via* the acetyl-CoA pathway. The fatty acids are subsequently oxidized at the terminal (ω) or subterminal (ω-1) position by a cytochrome P450 monooxygenase enzyme. The resulting hydroxylated fatty acids are β-glycosidically linked to a first glucose molecule at the 1'-position by a glycosyltransferase I. A second glucose molecule is linked to the 2'-position of the first one by a glycosyltransferase II, yielding sophorolipid acid **2**. Subsequently, the carbohydrate head can be acetylated at the 6'- and/or 6"-position by an acetyl-CoA dependent acetyl transferase. After excretion in the fermentation medium, lactonization can occur at the 4"-position by an extracellular esterase.[31] The major microbial product is the diacetylated, ω-1 hydroxylated, mono-unsaturated sophorolipid lactone **1**.

Most sophorolipid fermentations are run at a temperature between 25 to 30°C and a pH of 3.5. Sophorolipid synthesis starts in the stationary phase under nitrogen-limiting conditions and is very dependent on good aeration conditions. The highest yields are obtained when both a hydrophilic and a hydrophobic carbon source are present, but fermentation on only one type of carbon source is possible. After the fermentation, sophorolipids are extracted with ethyl acetate and washed with hexane to remove residual fatty acids. On larger scale, physical separation methods such as centrifugation are applied for the isolation. Lactonic sophorolipids were also purified by crystallization.[32]

The biggest bottleneck for the sophorolipid production process is the variability in the composition of the fermentation product. To optimize chemical modification procedures, one single compound is preferred as starting compound. If this is not the case, problems can arise along the modification

pathway. For example, in the synthesis of sophorolipid alkyl esters *via* alkaline alcoholysis of di-acetylated sophorolipid lactone, the products are precipitated in water after reaction. In some cases, precipitation does not occur, possibly due to the presence of a considerable fraction of sophorolipid acids or residual fatty acids. Therefore, it is of utmost importance to have access to selective organisms for the fermentation process or suitable purification procedures to obtain the desired sophorolipid compounds in high purity.[22, 33]



Scheme 2. Biosynthesis of sophorolipid derivatives

2.2. Physiological activity

2.2.1. Biodegradability and toxicity

The biodegradability of sophorolipids was determined *via* biological oxygen demand studies and the manometric respirometry method (OECD 301C and 301F method).[11, 34] They can be classified as readily biodegradable since 61% of the product was degraded after only 8 days of cultivation. The aquatic toxicity is subdivided in acute and chronic toxicity. The acute toxicity is determined as the EC_{50} after 48 h on *Daphnia magna*, *Tetrahymena thermophila* and *Pseudokirchneriella subcaptitata.*

This EC_{50} proved to be ten times higher as compared to conventional surfactants.[11, 35] The chronic toxicity was determined *via* a reproduction test on *Daphnia magna*. The no-observed-effect concentration (NOEC) was 11.3 µg/mL, which is also ten times higher as compared to conventional surfactants.[35] The cytotoxicity was determined by the MTT method with human epidermal keratinocytes, displaying a lower cytotoxicity for sophorolipids than for surfactin, arthrofactin, pluronic L31, sodium dodecyl sulfate and polyoxyethylene lauryl ether.[34] The cytotoxicity was also evaluated on normal Chang liver cells, displaying an IC_{50} value of 81900 µg/mL for crude sophorolipids and a higher cell viability for acidic sophorolipids compared to lactonic ones.[36] Sophorolipids are not irritating to skin and eyes, have an oral safety level which is greater than or equal to 5 mL/kg body weight and cause no allergic reactions.[37]

2.2.2. Dermatological activity

Sophorolipids inhibit elastase activity, the enzyme which is responsible for the degradation of elastin fibres, at a concentration of 50000 µg/mL and also inhibits radical effects towards the hydroxy-radical at a concentration of 830 µg/mL.[37] Diacetylated sophorolipid lactones stimulate the metabolism of skin dermis fibroblast cells and hereby the *in vitro* neosynthesis of collagen.[38] Therefore, they possess restructuring and tissue repairing activity which is higher as compared to crude sophorolipid mixtures. Besides moisturizing and skin-conditioning properties, sophorolipid lactones proved active to eliminate dandruff.[39] Moreover, sophorolipids possess desquamating, depigmenting and melanogenesis inhibiting activity.[40] The desquamation is achieved by detachment of the corneocytes and offers opportunities for the use of sophorolipids against acne and as an anti-wrinkle product. Sophorolipids induce the secretion of leptin by adipocytes, hereby inducing the lipolysis of adipocytes and reducing the subcutaneous deposition of fat.[41]

2.2.3. Antimicrobial activity

Diacetylated sophorolipid lactones synthesized by *Starmerella bombicola* inhibited the growth of *Candida*, *Pichia*, *Debaryomyces*, *Saccharomycopsis* and *Lodderomyces* yeasts on *n*-alkanes.[42] Natural sophorolipids also inhibited the growth of *Candida albicans*, *Candida Antarctica* and *Candida tropicalis* with respectively 30, 32 and 25% at a concentration of 5000 µg/mL, while the inhibition with sophorolipid acids was respectively 24, 45 and 40%.[43] Kulakovskaya *et al*. determined the minimum inhibitory concentrations (MIC) of sophorolipids against *Filobasidiella neoformans*, *Candida tropicalis* and *Candida albicans* as respectively 1000, 15000 and 15000 µg/mL.[44] The antibacterial activity of sophorolipids against *Rhodococcus erythropolis*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Moraxella* sp., *Pseudomonas putida*, *Enterobacter aerogenes* and *Escherichia coli* was evaluated, displaying a higher activity against Gram-positive bacteria than

Gram-negative bacteria.[45] The activity was expressed as minimum lethal doses (MLD_{50}) and was 98 µg/mL for *R. erythropolis*, *B. subtilis*, *S. agalactiae* and *Moraxella* sp., and >6250 µg/mL for *S. epidermis*, *P. putida*, *E. aerogenes* and *E. coli*. Kulakovskaya *et al*. determined the MIC values of sophorolipids against *E. coli*, *Streptococcus salivarius* and *Micrococcus luteus* as respectively 32000, 23000 and 23000 mg/mL.[44] More antimicrobial activities against other species are described in a patent by Gross and Shah.[46] Overall, sophorolipid lactones perform better than sophorolipid acids, with the lowest MIC values of 10 µg/mL for sophorolipid lactones against *Bacillus subtilis* and *Rhodococcus rhodochrous*. Sleiman *et al*. evaluated MIC values of different sophorolipid derivatives in sucrose and ethanol vehicles against *Escherichia coli* and *Staphylococcus aureus*.[47] No significant inhibitory activity was observed for most derivatives at a concentration of 512 µg/mL and against *S. aureus* at a concentration of 218 µg/mL for two derivatives in an ethanol vehicle. The antibacterial activity of lauryl alcohol based sophorolipids was also evaluated against *E. coli* and *S. aureus*, respectively displaying a zero percent survival at 3000 µg/mL after 2 h and at 600 µg/mL after 4 h.[48] Both crude and acidic sophorolipids inhibited the growth of *Cupriavidus necator* and *Bacillus subtilis* at a concentration of 50000 µg/mL.[49] They also disrupted biofilms of *B. subtilis* and a mixed culture of *B. subtilis* and *S. aureus* at a concentration of 50000 µg/mL. Synergistic effects were observed for administration of sophorolipids with the antibiotics tetracycline and cefaclor against respectively *S. aureus* and *E. coli*.[50] Sophorolipids inhibited the motility of the harmful algae *Alexandrium tamarense*, *Heterosigma akashiwo* and *Cochlodinium polykrikoides* at concentrations of 10-20 µg/mL.[51] At these concentrations, sophorolipids have low adverse effects on the non-harmful microalgae *Platymonas helgolandica* var. *tsingtaoensis*, *Isochrysis galbana* and *Nitzschia closterium* f. *minutissima*.[52] The EC_{50} for the zooplankton *Strombidium* sp., *Calanus sinicus* and *Neomysis awatschensis* was respectively 20, 50 and 150 µg/mL after 96 h. The EC_{50} for the zooplankton *Artemia salina* was 600 µg/mL after 24 h. The fish *Lateolabrax japonicas* and *Paralichthys olivaceus* displayed an EC_{50} of respectively 60 and 110 µg/mL and the relative clearance rate of the mussel *Mytilus edulis* decreased to 80% at a sophorolipid concentration of 20 µg/mL. Synergistic effects were observed for the combination of sophorolipids and loess on the motility inhibition and removal of the harmful algae *C. polykrikoides* and *A. tamarense*.[53] It can be concluded that sophorolipids display no significant antibacterial activity against clinically relevant bacteria, but are effective in the inhibition of harmful algae in concentrations which are not detrimental to most of the tested organisms.

2.2.4. Anticancer activity

Sophorolipids induced differentiation of human acute promyelocytic leukemia cell line HL60.[54] At a concentration of 10 µg/mL, the proliferation of the HL60 cells and the protein kinase C activity was inhibited after 2 days and differentiation into monocytes took place. For two other leukemic cell

lines, the human myelogenous leukemia cell line K562 and the human basophilic leukemia cell line KU812, differentiation into megakaryocytes was also induced at a sophorolipid concentration of 15 µg/mL. Chen *et al*. evaluated the anticancer activity of diacetylated sophorolipid lactone on liver cancer cell line H7402, lung cancer cell line A549 and leukemia cell lines HL60 and K562 *via* an MTT assay.[55] The cell proliferation of all four cell lines was inhibited at concentrations ranging up to 62.5 µg/mL after 2 days with 0% cell viability at concentrations exceeding 62.5 µg/mL. The mechanism of anticancer activity on the liver cancer cell line H7402 was identified as apoptosis.[56] The condensation of chromatin, nuclear fragmentation and appearance of apoptotic bodies was observed. At a sophorolipid concentration of 50 µg/mL, cell viability of both liver cancer cell line H7402 and lung cancer cell line A549 was completely inhibited while only a little decrease was observed for the normal liver cell lines HL7702 and Chang liver. The anticancer activity of diacetylated sophorolipid lactone and sophorolipid acid against human pancreatic cancer cells was also evaluated.[57] A decreasing cytotoxicity of respectively 40.3 to 3.4% and 49 to 0% was observed with increasing concentration from 500 to 2000 µg/mL. No cytotoxicity was observed against healthy peripheral blood mononuclear cells. Shao *et al*. described the anticancer activity of different sophorolipid derivatives against the esophageal cancer cell lines KYSE109 and KYSE450.[58] Diacetylated sophorolipid lactone displayed the highest activity with complete inhibition of both cell lines at a concentration of 30 µg/mL. The anticancer activity depended on the acetylation and unsaturation degree of the derivatives. No inhibition was observed for acidic sophorolipids. Evaluation of the anticancer activity on MDA-MB-321 breast cancer cell line demonstrated a higher activity for sophorolipid lactones compared to sophorolipid acids, with IC_{50} values of respectively 15-20 and 80 µg/mL.[59] Sophorolipids also induce differentiation in LN-229 glioma cell lines.[60] Morphological changes were detected at concentrations of 10 µg/mL and 0.4 µg/mL for respectively oleic acid and linolenic acid based sophorolipids.

2.2.5. Immunoregulatory activity

Sophorolipids possess a pro-inflammatory activity by activating macrophages to induce the release of cytokines.[40] They also promote wound healing due to the proliferative effect of these cytokines on the fibroblasts, the phagocytosis of bacteria, cells and cellular fragments which could obstruct the wound by macrophages and their fibrinolytic activity. Sophorolipids can reduce septic shock related mortality by inhibition of the nitrogen oxide production of macrophages and modulation of the inflammatory response.[61] Experiments were performed in *in vivo* rat models with intravenous and intraperitoneal administration of Nembutal to induce septic shock. The survival rate after 36 h with and without addition of 5 mg/kg natural sophorolipid mixture was respectively 81.8 and 47.8% for the intervenous administration and 67 and 53% for the intraperitoneal administration. Macrophages

which were *in vitro* cultured with lipopolysaccharides showed reduced nitrogen oxide production and induced expression of several cytokines. The viability of these macrophages also increased from 56 to 66% and from 23 to 36% after respectively 36 and 48 h.[62] Moreover, sophorolipids at a concentration of 100 µg/mL decreased the IgE production in U266 myeloma cells with 63%.[63] The effect of dosing was evaluated in the *in vivo* rat models.[64] When a single dose was administered, survival increased with 28 and 42% after respectively 24 and 72 h while survival increased with 39 and 26% for sequential dosing after respectively 24 and 72 h. Increased mortality was observed for treatment with purified diacetylated sophorolipids lactone. Sophorolipids also reduced asthma severity in an *in vivo* asthma model with mice, which was demonstrated on the basis of decreased leukocytic infiltrate in the lungs and decreased levels of ovalbumin specific IgE in the bronchoalveolar lavage fluid.[65]

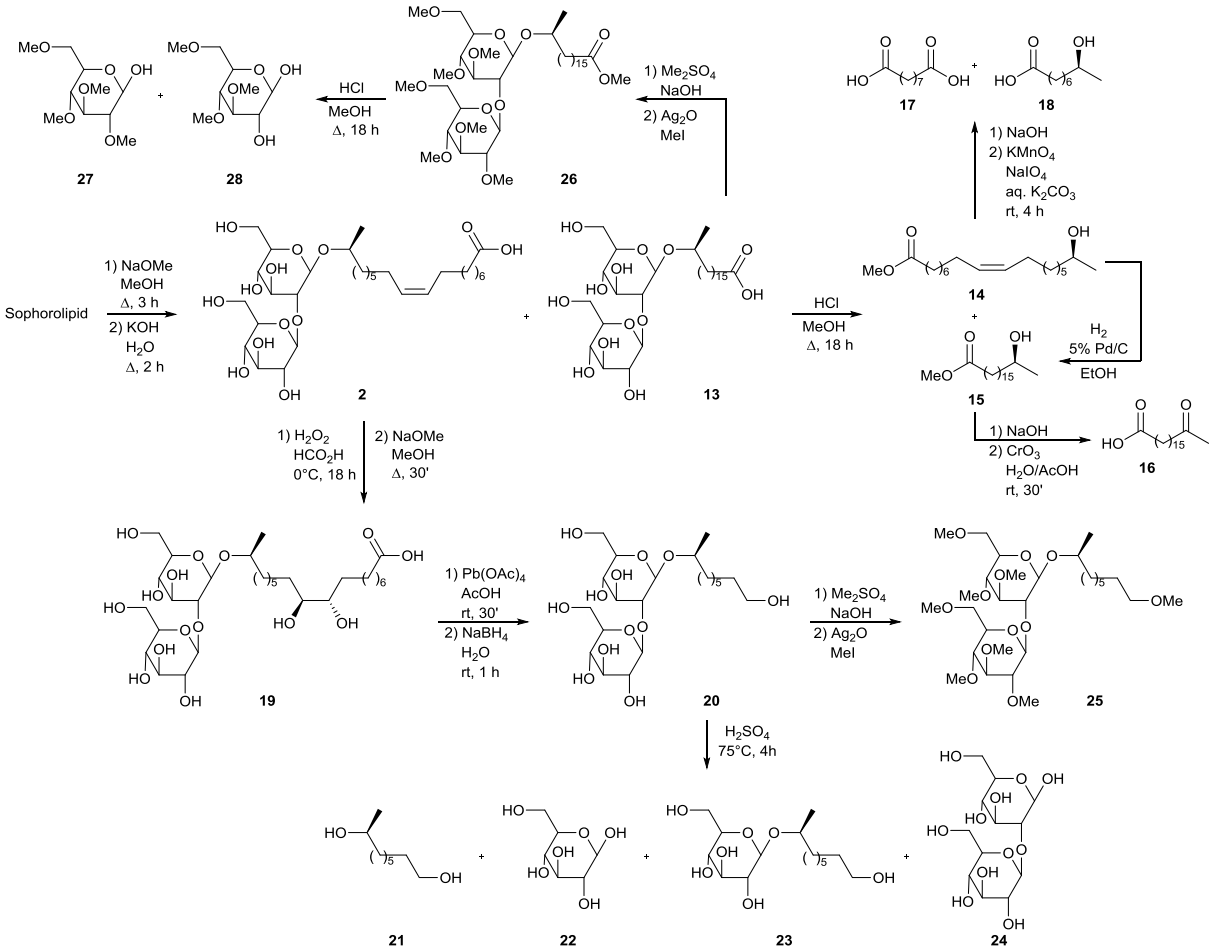
2.2.6. Spermicidal and antiviral activity

Sophorolipids are active as spermicidal and antiviral agents.[66-67] Natural mixture sophorolipids and sophorolipid lactones displayed a minimum effective concentration of respectively 800 and 1000 µg/mL after 30 seconds of incubation. Sophorolipids at a concentration of 300 µg/mL immobilized spermatozoa completely and irreversibly after 2 minutes. Immobilization occurred faster than death and the mechanism of action is most likely membrane perturbation and disruption. Sophorolipids also displayed some degree of anti-HIV activity, with sophorolipid acids being more potent than sophorolipid lactones. Natural mixture sophorolipids and sophorolipid lactones exerted high cytotoxicity against human vaginal cells with a 50% effective concentration around 15 µg/mL, while sophorolipid acids displayed a 50% effective concentration higher than 100 µg/mL. Natural mixture sophorolipids and sophorolipid lactones also induced the production of pro-inflammatory cytokines by vaginal epithelial cells. Sophorolipids also displayed anti-herpes virus activity for which the Epstein-Bar virus was used as a model organism.[68] The EC_{50} values for diacetylated sophorolipid lactone and sophorolipid acid were respectively 37.5 and 79.1 µg/mL (25.8 and 49.2 µM).

2.3. Modifications in the context of structural characterization

The first modifications of sophorolipids produced by *Candida magnoliae* (formerly known as *Torulopsis magnoliae*) are described by Gorin *et al*. in the context of the characterization of this class of biosurfactants (Scheme 3).[69] In a first step, the crude sophorolipid product was transformed into unsaturated sophorolipid acid **2** and saturated sophorolipid acid **13** through removal of the acetyl groups with sodium methoxide. Acid methanolysis of sophorolipid acids **2** and **13** resulted in the cleavage of fatty acid methyl esters **14** and **15** which were separated *via* fractional crystallization. The saturated methyl ester **15** was oxidized with chromium trioxide into 17-oxo-stearic acid **16**, which enabled the determination of the hydroxyl position. The unsaturated methyl ester **14** was oxidized

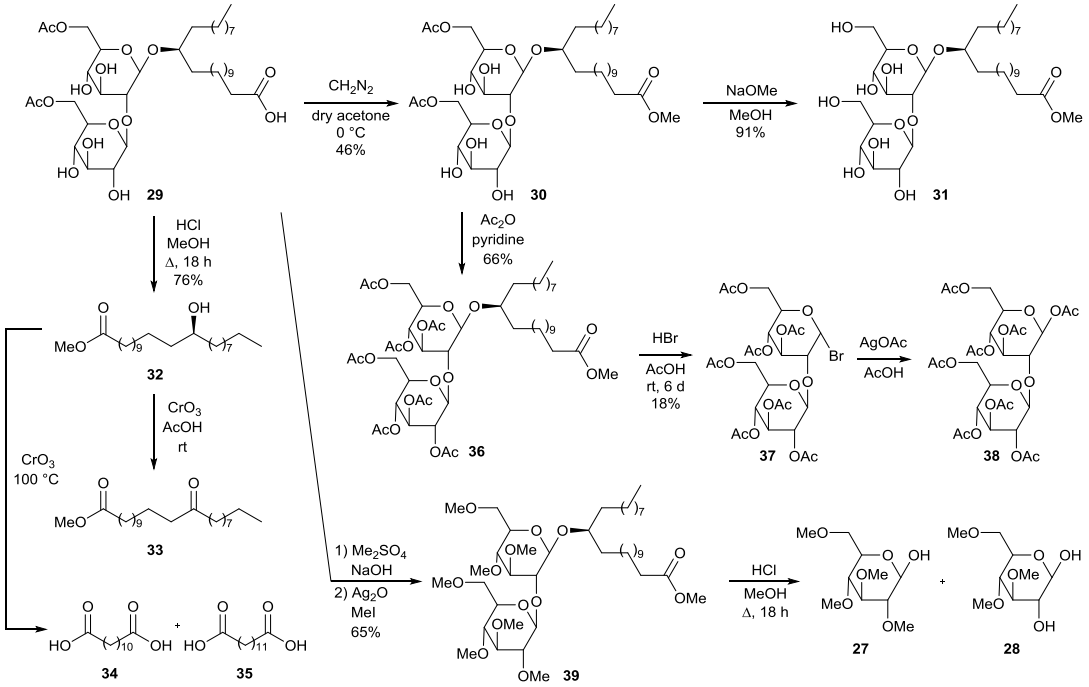
with potassium permanganate and sodium periodate into azelaic acid **17** and 8-hydroxy-pelargonic acid **18**, which enabled the determination of the double bond. Unsaturated sophorolipid acid **2**, on the other hand, was oxidized with performic acid followed by alkaline methanolysis into the *threo*-9,10-dihydroxy sophorolipid **19**. Subsequent oxidation with lead(IV) acetate and reduction with sodium borohydride yielded sophorolipid alcohol **20**. Partial acid hydrolysis furnished a mixture of 1,8-*S*-nonanediol **21**, glucose **22**, 1,8-*S*-nonanediol-β-*D*-glucopyranoside **23** and sophorose **24**. The latter two were isolated by chromatography. Sophorolipid alcohol **20** and saturated sophorolipid acid **13** were methylated into respectively sophorolipid ether **25** and sophorolipid methyl ester **26**. Both compounds were hydrolyzed into 2,3,4,6-tetra-*O*-methyl-*D*-glucose **27** and 3,4,6-tri-*O*-methyl-*D*-glucose **28** which confirmed the 1,2-glycosidic link between the two glucose units.



Scheme 3. Sophorolipid modifications in the context of the structure determination

Similar modifications were performed by Tulloch *et al*. for the characterization of diacetylated sophorolipid **29** which is produced by *Rhodotorula bogoriensis* (Scheme 4).[70] In a first step, sophorolipid **29** was transformed into diacetylated sophorolipid methyl ester **30** with diazomethane. Alkaline methanolysis furnished the corresponding methyl ester **31**. Acid methanolysis of sophorolipid acid **29** resulted in the cleavage of fatty acid methyl ester **32**. Oxidation with chromium

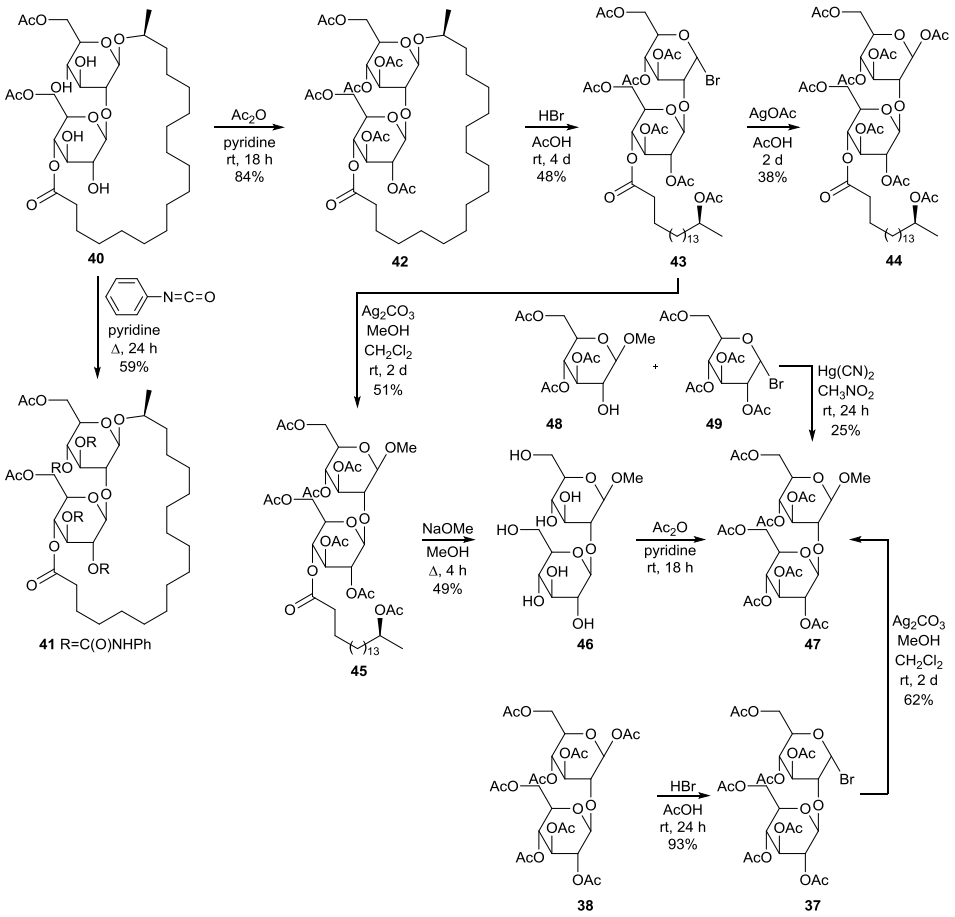
oxide at room temperature or 100°C yielded respectively methyl 13-oxodocosanoate **33** or dodecanedioic acid **34** and tridecanedioic acid **35**, which enabled the determination of the hydroxyl position. Diacetylated sophorolipid **30** was acetylated with acetic anhydride towards the peracetylated sophorolipid **36**. Bromination towards α-acetobromosophorose **37** was followed by the synthesis of β-octaacetyl sophorose **38**. Diacetylated sophorolipid **39** was further methylated towards sophorolipid methyl ester **29** and subsequently hydrolyzed into 2,3,4,6-tetra-*O*-methyl-*D*-glucose **27** and 3,4,6-tri-*O*-methyl-*D*-glucose **28** which confirmed the 1,2-glycosidic link between the two glucose units.



Scheme 4. Modifications for the structural determination of diacetylated sophorolipid 29

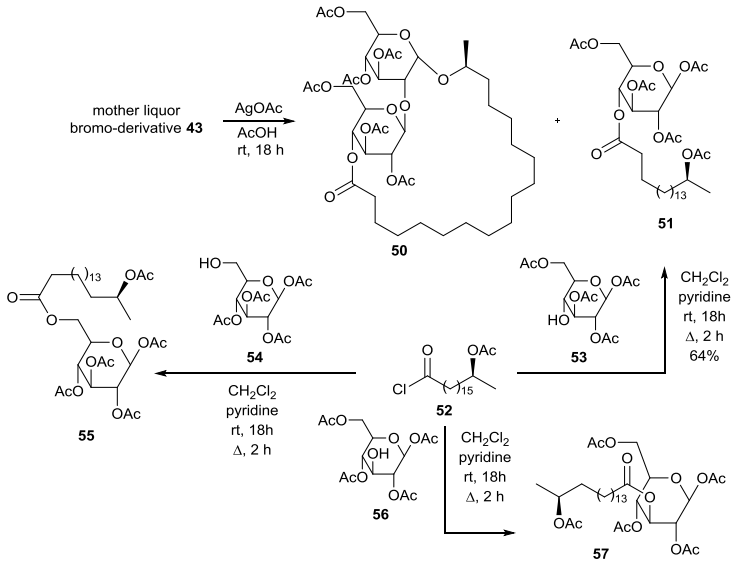
Modifications starting from diacetylated sophorolipid lactone **40** are described by Tulloch *et al.* (Scheme 5).[71] Diacetylated sophorolipid lactone **40** proved to be the major fermentation product and was obtained in pure form after crystallization and hydrogenation of the fermentation product followed by subsequent chromatography. Tetraphenylurethane sophorolipid **41** was synthesized *via* reaction with phenyl isocyanate. Acetylation of diacetylated sophorolipid lactone **40** was performed with acetic anhydride towards the hexaacetylated sophorolipid lactone **42** followed by brominolysis towards α-bromosophorose hexaacetate derivative **43**. Subsequent treatment with silver acetate gave the corresponding β-acetoxysophorose derivative **44**. The α-bromosophorose hexaacetate **43** was converted into β-methoxysophorose hexaacetate **45** followed by deacetylation towards β-methoxysophorose **46** and acetylation towards β-methoxysophorose heptaacetate **47**. The latter compound was also prepared *via* brominolysis of β-sophorose octaacetate **38** towards β-bromosophorose heptaacetate **37** and subsequent reaction with methanol in the presence of silver

carbonate. A third method for the synthesis of β-methoxysophorose heptaacetate **47** comprises the Koenigs-Knorr reaction of 3,4,6-tri-*O*-acetyl-β-*D*-glucopyranoside **48** and α-acetobromoglucose **49** in the presence of mercuric cyanide in nitromethane. The brominolysis reaction of hexaacetylated sophorolipid lactone **42** also resulted in the cleavage of the linkage between the two glucose units and reversion of the anomeric center.



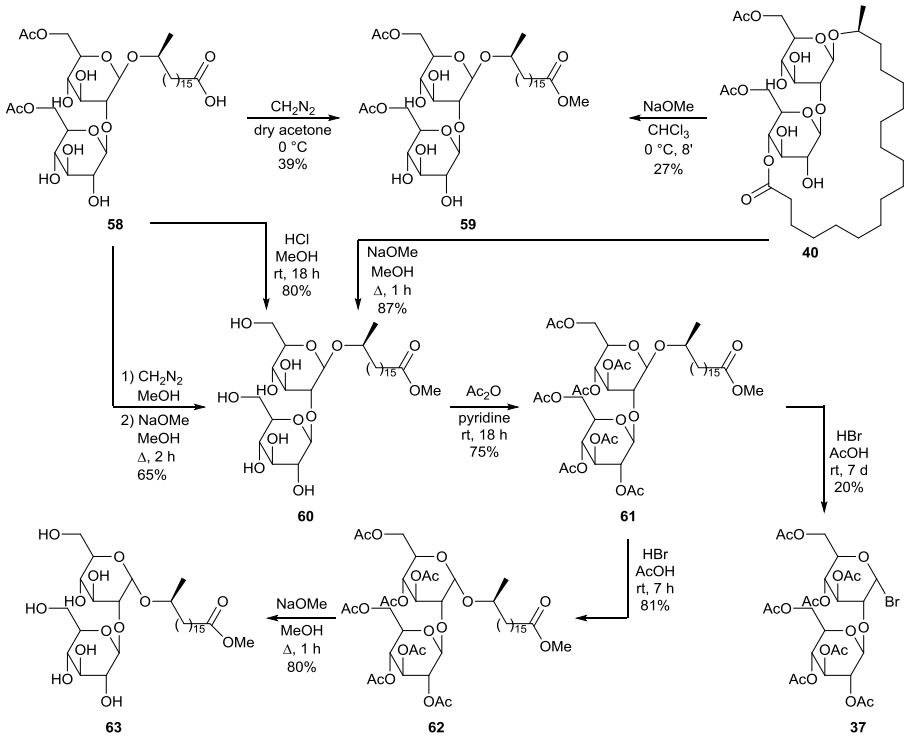
Scheme 5. Modifications of diacetylated sophorolipid lactone 40

Treatment of the mother liquor after isolation of α-bromosophorose hexaacetate **43** with silver acetate and subsequent chromatography resulted in the isolation of hexaacetylated α-sophorolipid lactone **50** and β-glucose tetraacetate derivative **51** (Scheme 6). The structure of the latter compound was confirmed upon reaction of the acid chloride **52** synthesized from 17-*L*-acetoxyoctadecanoic acid and 1,2,3,6-tetra-*O*-acetyl-β-*D*-glucopyranose **53**. In this context, acid chloride **52** was also coupled with 1,2,3,4-tetra-*O*-acetyl-β-*D*-glucopyranose **54** and 1,2,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose **56** towards the β-glucose tetraacetate derivatives **55** and **57** respectively. Isolation and characterization of β-glucose tetraacetate derivative **51** demonstrated that the fatty acid tail is linked to the C4”-position in the diacetylated sophorolipid lactone **40**.



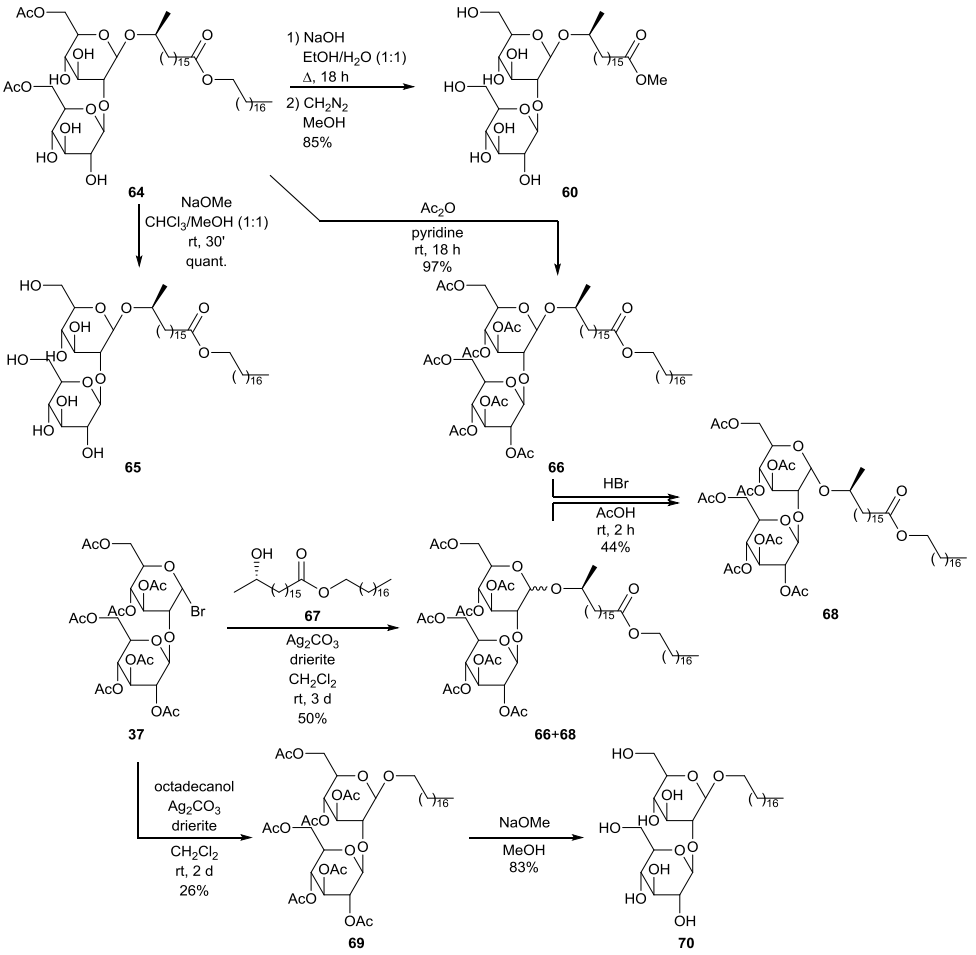
Scheme 6. Isolation of brominolysis side-products

Next to diacetylated sophorolipid lactone **40**, a second major fermentation product was present, i.e. diacetylated sophorolipid acid **58**. Treatment of sophorolipid acid **58** with diazomethane on the one hand, and treatment of sophorolipid lactone **40** with low concentrated sodium methoxide on the other hand both resulted in the synthesis of diacetylated sophorolipid methyl ester **59** (Scheme 7). Subsequent deacetylation gave sophorolipid methyl ester **60**, which was also prepared by deacetylation of sophorolipid lactone **40** and by treatment of sophorolipid acid **58** with a methanolic hydrogen chloride solution. The syntheses of sophorolipid methyl esters **59** and **60** confirmed that the structure of diacetylated sophorolipid acid **58** is similar to that of diacetylated sophorolipid lactone **40**. Sophorolipid methyl ester **60** was acetylated towards heptaacetylated sophorolipid methyl ester **61** followed by brominolysis towards α-acetobromosophorose **37**. When the reaction time was reduced to seven hours, heptaacetylated α-sophorolipid methyl ester **62** was obtained, which confirmed that brominolysis reaction conditions induced reversion of the anomeric center. Deacetylation with sodium methoxide gave α-sophorolipid methyl ester **63**.



Scheme 7. Modifications of diacetylated sophorolipid acid 58

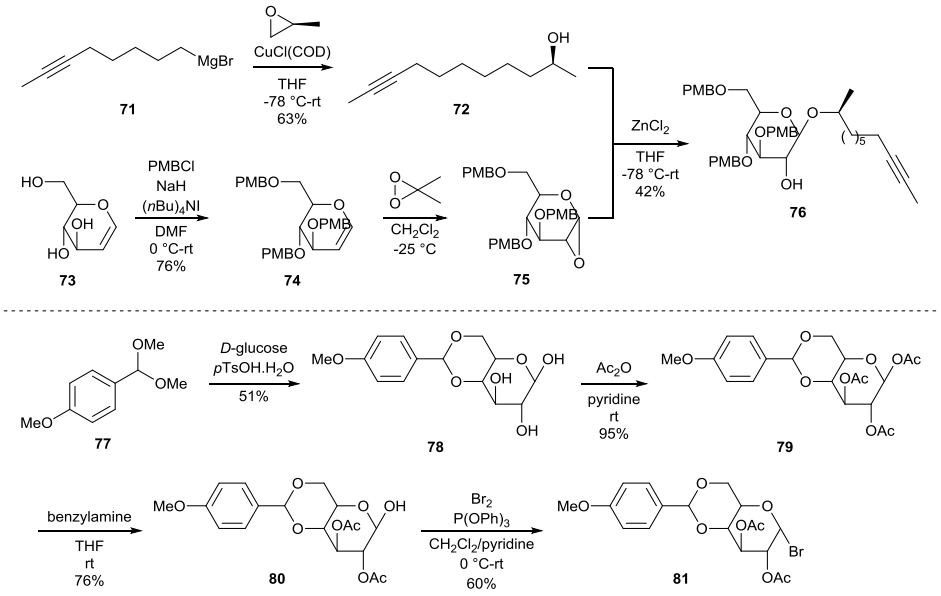
The unexpected production of diacetylated sophorolipid octadecyl ester **64** *via* fermentation of octadecanol by *Starmerella bombicola* and subsequent modifications is described by Tulloch and Spencer (Scheme 8).[72] Alkaline hydrolysis with sodium hydroxide followed by reaction with diazomethane in methanol yielded sophorolipid methyl ester **60**. Methanolysis with sodium methoxide gave sophorolipid octadecyl ester **65** and acetylation resulted in the synthesis of heptaacetylated sophorolipid octadecyl ester **66**. To confirm the structure of diacetylated sophorolipid octadecyl ester **64**, synthesis of heptaacetylated sophorolipid octadecyl ester **66** *via* the Koenigs-Knorr reaction was attempted. Octadecyl 17-*L*-hydroxyoctadecanoate **67** was synthesized from 17-*L*-formyloxyoctadecanoyl chloride and octadecanol and subsequently reacted with α-acetobromosophorose **37**. A mixture of α and β anomers **68** and **66** was obtained which was completely converted into heptaacetylated α-sophorolipid octadecyl ester **68** upon reaction with hydrogen bromide. Synthesis of the heptaacetylated derivative **69** of the desired fermentation product, *i.e.* an octadecyl β-sophoroside, was performed *via* the Koenigs-Knorr reaction of octadecanol with α-acetobromosophorose **37**. Deacetylation gave octadecyl β-sophoroside **70**.



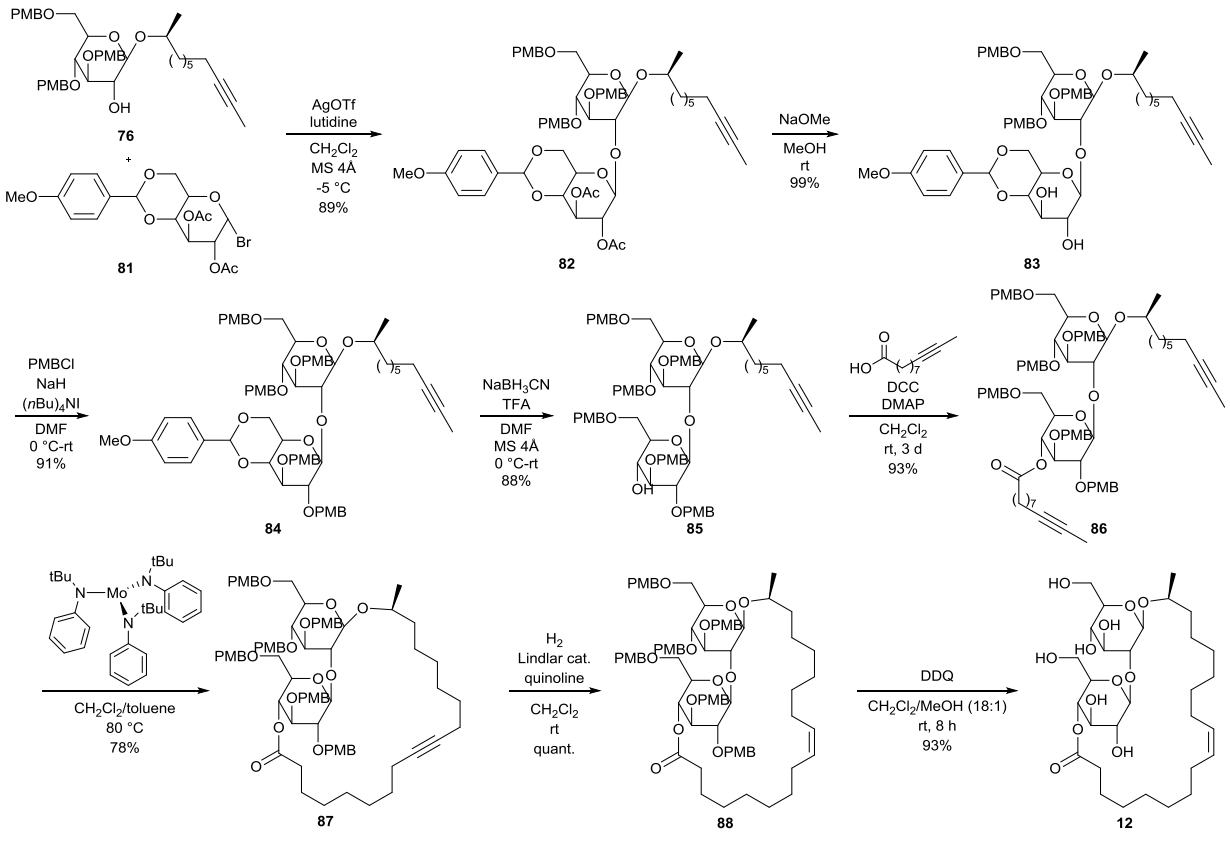
Scheme 8. Modifications of diacetylated sophorolipid octadecyl ester 64

The first total synthesis of sophorolipid lactone **12** was described by Fürstner *et al*.[73] In a first step, two major building blocks were synthesized (Scheme 9). The ring-opening reaction of (*S*)-propenoxide with Grignard reagent **71** yielded the enantiomerically pure alcohol **72**. *D*-glucal **73** was protected with *p*-methoxybenzyl chloride and the resulting tri-*O*-PMB ether **74** was subsequently treated with dimethyldioxirane to yield epoxide **75**. Alcohol **72** was reacted with epoxide **75** into the first building block **76**. On the other hand, *D*-glucose and *p*-methoxybenzaldehyde dimethylacetal **77** were coupled *via* a transacetalization reaction to yield 4,6-*O*-*p*-methoxybenzylidene acetal **78**. Peracetylation towards acetal **79** was followed by selective deprotection of the anomeric center yielding reducing sugar **80**. Reaction with bromine delivered the second building block **81**. The two building blocks were coupled *via* a glycosylation reaction under modified Koenigs-Knorr conditions which yielded the desired sophorose glycoside **82** (Scheme 10). Deacetylation into diol **83** was followed by protection with *p*-methoxybenzyl chloride. The resulting PMB ether derivative **84** was treated with sodium cyanoborohydride to remove the acetal protecting group. The *p*-methoxybenzylated sophorolipid **85** was esterified with 9-undecynoic acid towards diyne **86**. A ring-closing metathesis reaction was performed which was catalyzed by a molybdenum catalyst to

yield cycloalkyne **87**. Protected sophorolipid lactone **88** was obtained *via* Lindlar hydrogenation and subsequent deprotection delivered the desired sophorolipid lactone **12**.



Scheme 9. Synthesis of building blocks 76 and 81

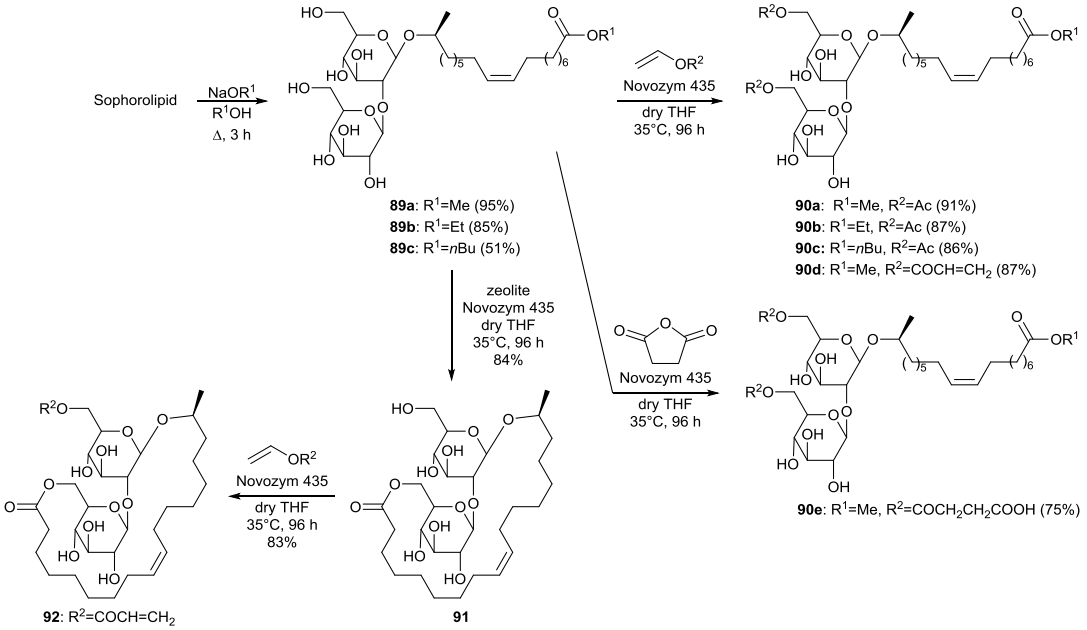


Scheme 10. First total synthesis of sophorolipid lactone 12

2.4. Modifications towards new derivatives

2.4.1. Regioselective reactions at the sugar head group

Regioselective acylations of the sophorolipid head group through the use of enzymes is described by Bisht *et al.* (Scheme 11).[74] First, the crude sophorolipid fermentation product needed to be transformed into a single pure compound to enable the synthesis of well-defined sophorolipid analogues. Therefore, the starting product was subjected to alkaline alcoholysis with sodium alkoxides, yielding sophorolipid esters **89**. Transformation of the starting product into sophorolipid acid was not useful, since this acid is only soluble in water and polar aprotic solvents which are not suitable for transesterification reactions with lipase enzymes. The sophorolipid esters **89** were subjected to lipase-catalyzed esterifications with an excess of vinyl acetate, vinyl acrylate or succinic anhydride. Multiple enzymes were evaluated and the highest conversion was obtained with Novozym 435. With this enzyme, selective acylations at both the 6'- and 6"-position of the sugar head were obtained, yielding acylated sophorolipid esters **90**. Acylation of only one of these positions was attempted through variation of the ratio sophorolipid ester/acylating agent. With a ratio of 1:1 or less, 1,6"-sophorolipid lactone **91** was formed which is an unnatural analogue of the 1,4"-sophorolipid lactone. This lactone was subsequently used for the regioselective esterification at the 6'-position of the sugar head, yielding the monoacrylated sophorolipid lactone **92**.

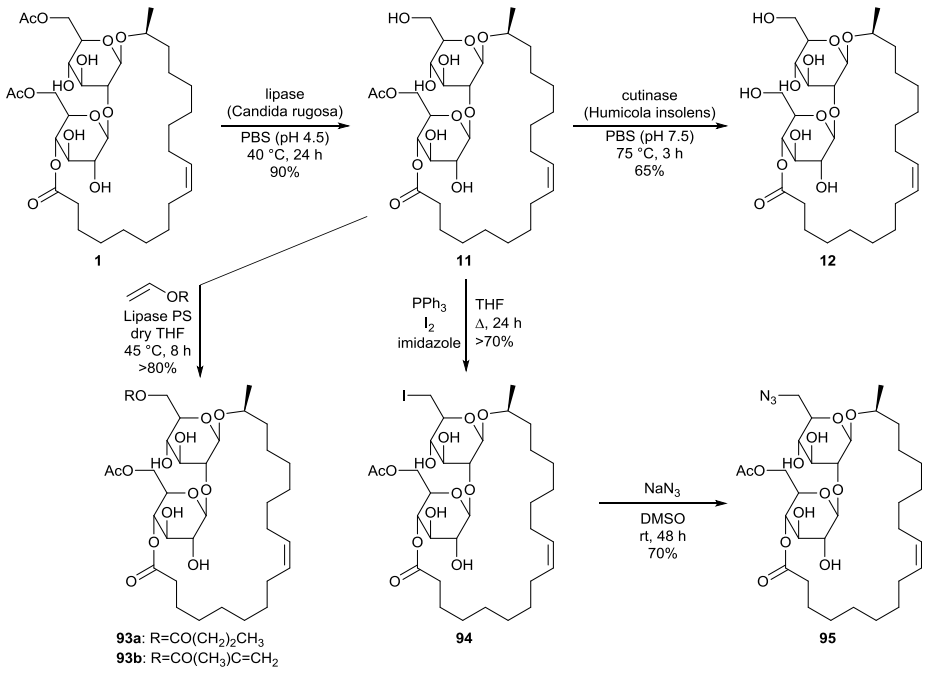


Scheme 11. Regioselective acylation of sophorolipid esters 89 at both the 6'- and 6"-position

Sophorolipid methyl ester **89a** displayed a cytotoxicity of 63 ± 5% against human pancreatic cells at a concentration ranging from 500 to 2000 µg/mL.[57] Sophorolipid ethyl esters **89b** and **90b** displayed a lower cytotoxicity of respectively 23.6% at 1000 µg/mL and 42.6% at 500 µg/mL. No cytotoxicity was

observed against healthy peripheral blood mononuclear cells. Sophorolipid ethyl ester **90b** also reduced septic shock related mortality in *in vivo* rat models with 23% and possessed high spermicidal and anti-HIV activity comparable to those of nonoxynol-9.[64, 67] Unfortunately, high cytotoxicity was exerted on human vaginal cells.

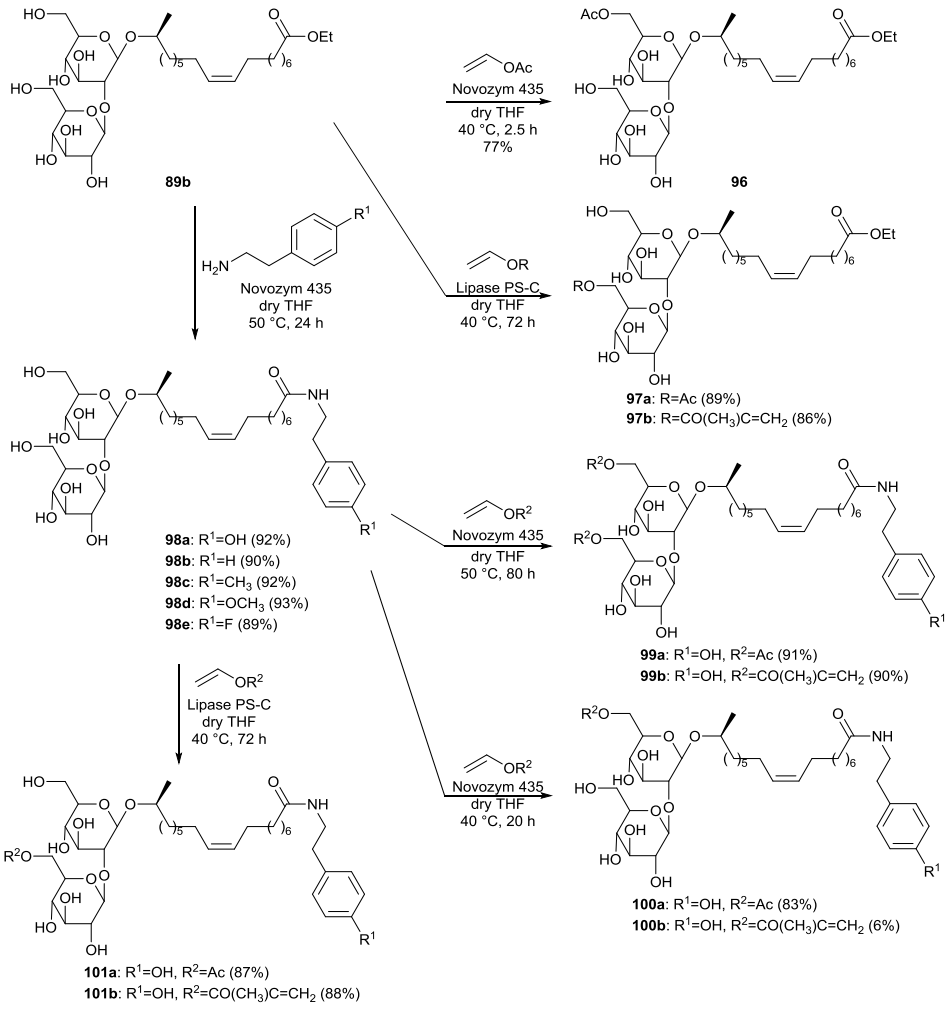
Selective enzyme-catalyzed deacetylation of sophorolipid lactone **1** at the 6'-position was performed by Peng *et al*. with the lipase from *Candida rugosa* (Scheme 12).[75] This mono-acetylated sophorolipid lactone **11** was subsequently hydrolyzed into the non-acetylated sophorolipid lactone **12** with the cutinase from *Humicola insolens*. Methacrylate and butyrate groups were introduced at the 6'-position through an enzyme-catalyzed acylation of the mono-acetylated sophorolipid lactone **11** with the respective vinyl acylates. An azide group was introduced in two steps. First, a iodination was performed followed by reaction with sodium azide. Methacrylate and azide groups offer the opportunity to introduce bioactive groups through click reactions.



Scheme 12. Regioselective functionalization of sophorolipid lactone 1

More enzyme-mediated regioselective acylations of sophorolipids at the 6'- and/or 6"-position of the sugar head are described by Singh *et al.* (Scheme 13).[76] Selective acylation at the 6'-position was accomplished through reaction of sophorolipid ethyl ester **89b** with excess vinyl acetate and Novozym 435, yielding sophorolipid monoacetate **96**. A small amount of the corresponding sophorolipid diacetate was formed as well. When Lipase PS is used as catalyst, selective acylation at the 6"-position is obtained. Vinyl acetate and vinyl methacrylate were used respectively for the synthesis of sophorolipid monoacetates **97a** and **97b**. Enzymatic catalysis was also evaluated for the amidation of sophorolipid ethyl ester **89b** with a number of primary amines. Different enzymes were

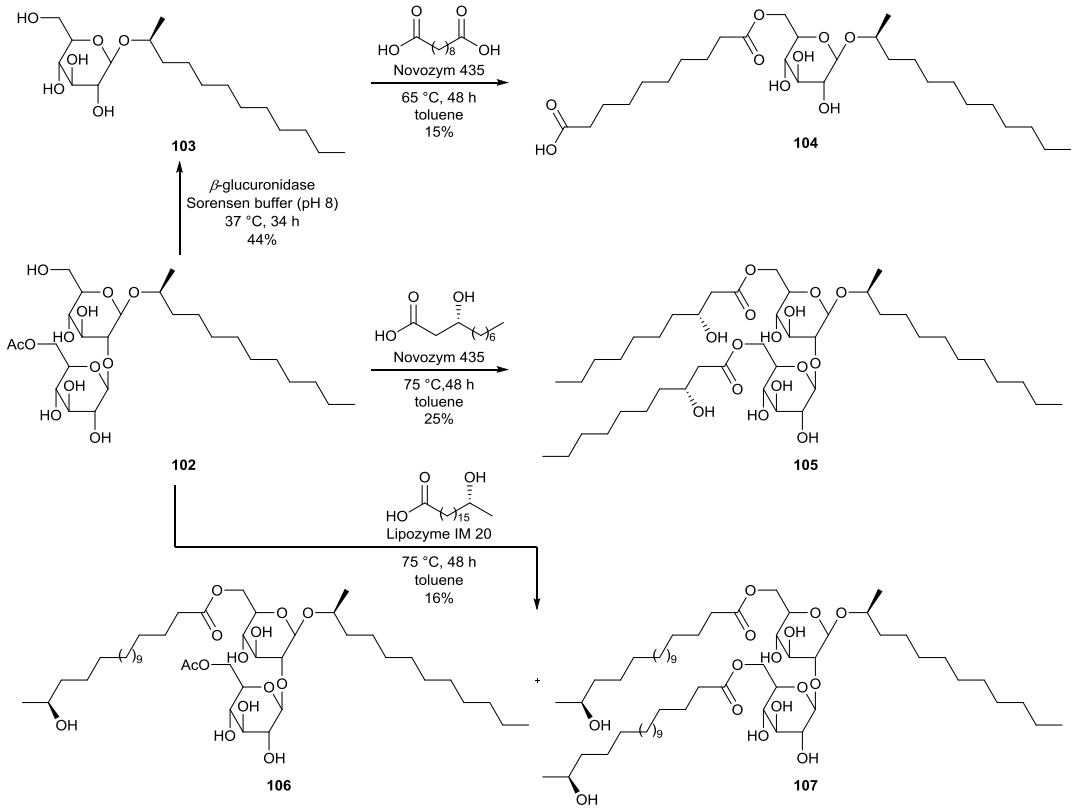
evaluated, of which only Novozym 435 was successful for the formation of sophorolipid amides **98** in high yield. The regioselective acylations with vinyl acetate and vinyl methacrylate at the 6'- and/or 6"-positions of the sugar head were extended to the sophorolipid amides, yielding sophorolipid diacetate **99** and sophorolipid monoacetates **100** and **101**. Also a one-pot synthesis of compound **99b** was successfully accomplished.



Scheme 13. Regioselective acylation and enzyme-catalyzed amidation of sophorolipid esters 89

The modification of 2-dodecyl sophorolipid **102**, prepared by fermentation with 2-dodecanol as substrate, was performed by Recke *et al*. (Scheme 14).[77] The 2-dodecyl sophorolipid **102** was transformed into the 2-dodecyl glucolipid **103** *via* the selective removal of a glucose unit with the β-glucuronidase enzyme. Subsequently, this glucolipid was acylated at the 6'-position with sebacic acid towards glucolipid **104**. The free carboxylic acid function offers the opportunity for further derivatization towards polyesters. The 2-dodecyl sophorolipid **102** was also acylated at the 6'- and 6"-position with the unusual fatty acids (*R*)-3-hydroxydecanoic acid and (*S*)-17-hydroxystearic acid which are obtained *via* hydrolysis of respectively rhamnolipids and sophorolipids. Acylation with (*R*)-3-hydroxydecanoic acid resulted in the synthesis of one major product, *i.e*. the diacylated

sophorolipid **105**. On the other hand, acylation with (*S*)-17-hydroxystearic acid was not complete after 48 hours, resulting in the formation of both the mono-acylated sophorolipid **106** and the di-acylated sophorolipid **107**. Longer reaction times were however not evaluated to obtain full conversion.

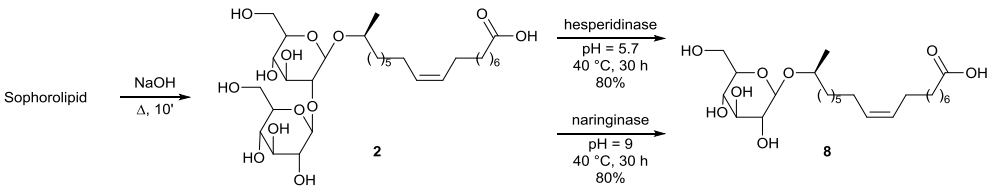


Scheme 14. Acylations at the 6'- and 6”-position of sophorolipid 102 and glucolipid 103

The emulsion stability, CMC values and reduction of the air-water surface tension (γ) and water-*n*-hexadecane interfacial tension (σ) of the derivatives were evaluated. A good stabilization of w/o emulsions was achieved with all derivatives. This was not the case for o/w emulsions. CMC, γ- and σ-values were respectively 150-200 mg/L, 27-50 mN/m and 3-7 mN/m. Antimicrobial activities of the derivatives were also evaluated. All derivatives showed strong growth inhibition against the Gram-positive bacteria *Bacillus megaterium* and *Bacillus subtilis*, and against the fungus *Candida magnoliae*. Only di-acylated sophorolipid **105** and the mixture of mono-acylated sophorolipid **106** and di-acylated sophorolipid **107** showed inhibition against the Gram-positive bacteria *Staphylococcus capitis*. No growth inhibition was observed against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, the fungi *Eurotium repens*, *Mycotypha microspora* and *Ustilago maydis*, and against the alga *Chlorella fusca*. Finally, the anti-tumor promoting activity of the derivatives was evaluated *via* a short-term *in vitro* assay for Epstein-Barr virus activation in Raji cells induced by TPA using heptyl-galactosyl-glyceride as a reference compound. Concentrations were expressed as mol ratio of compound compared to TPA. At 1000 mol ratio/TPA, only 10-15%

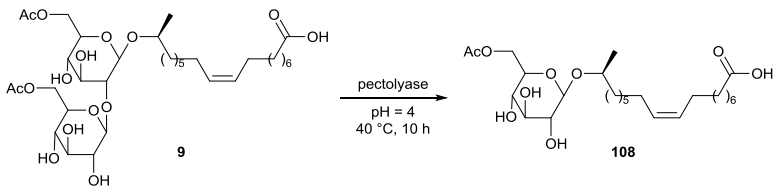
activation was observed for the derivatives. All compounds displayed a cell viability of 70% at this concentration, which corresponds to a weak cytotoxicity against the Raji cells.

The enzymatic conversion of crude sophorolipid fermentation product into glucolipid **8** is described by Rau *et al*. (Scheme 15).[78-79] In a first step, the crude fermentation product was transformed *via* alkaline hydrolysis into deacetylated sophorolipid acid **2**. Different glycosidases were evaluated for the specific release of one glucose molecule and the best results were obtained with the hesperidinase and naringinase enzyme. A comparison was made for the interfacial activities of glucolipid **8** and sophorolipid lactone both at the air-water and water-*n*-hexadecane interface. In both cases, the interfacial activity of the sophorolipid lactone could not be matched by glucolipid **8**.



Scheme 15. Deglycosylation of sophorolipid acid 2

A similar deglycosylation of diacetylated sophorolipid acid **9** is described by Imura *et al*. (Scheme 16).[80] For this acetylated substrate, no activity was observed with hesperidinase or naringinase as was the case for the non-acetylated compound. Selective cleavage of the β-1,2-glycosidic bond was obtained with invertase, pectinase solution, pectinase and pectolyase. The highest activity for the conversion towards acetylated glucolipid acid **108** was obtained with pectolyase. Evaluation of the surface active properties of sophorolipid acid **2**, glucolipid acid **8**, diacetylated sophorolipid acid **9** and acetylated glucolipid acid **108** showed little variation in CMC value for the different compounds.



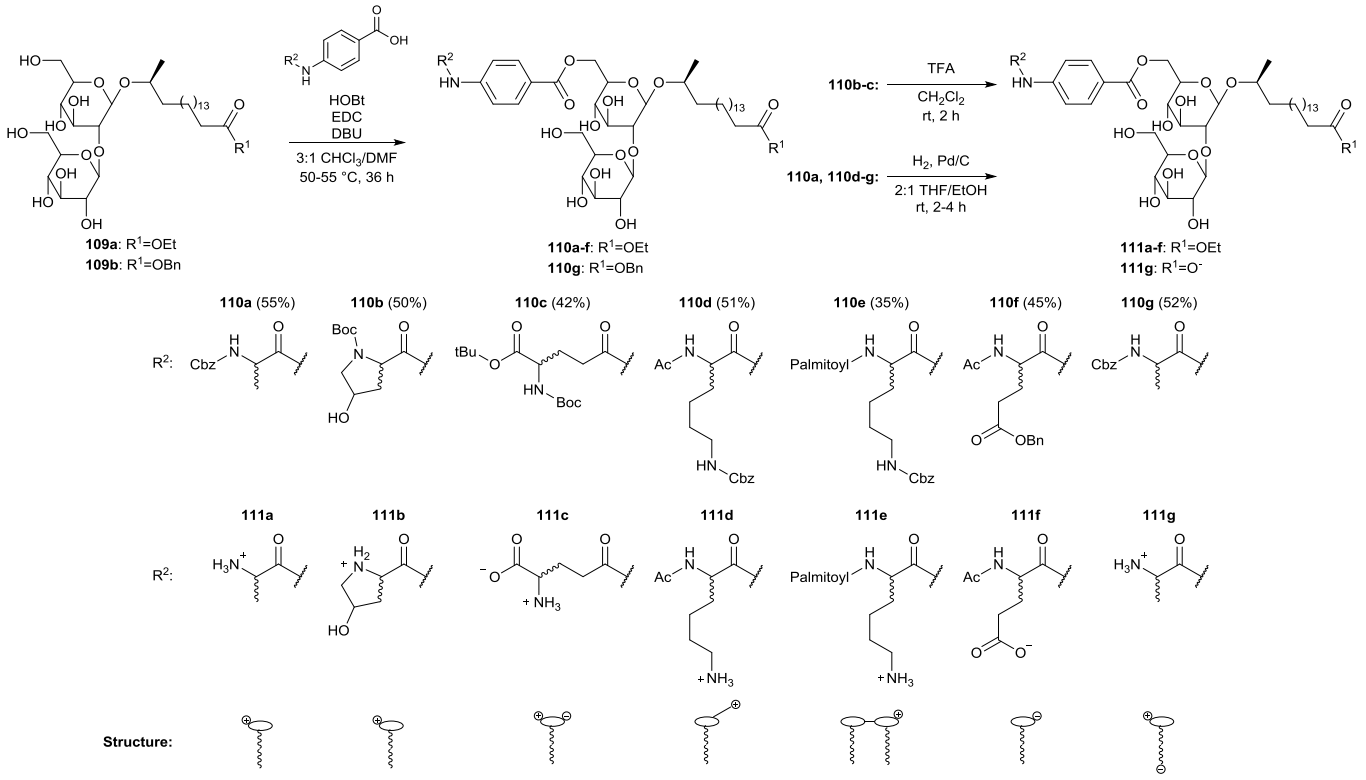
Scheme 16. Deglycosylation of diacetylated sophorolipid 9

2.4.2. Non-selective reactions at the sugar head

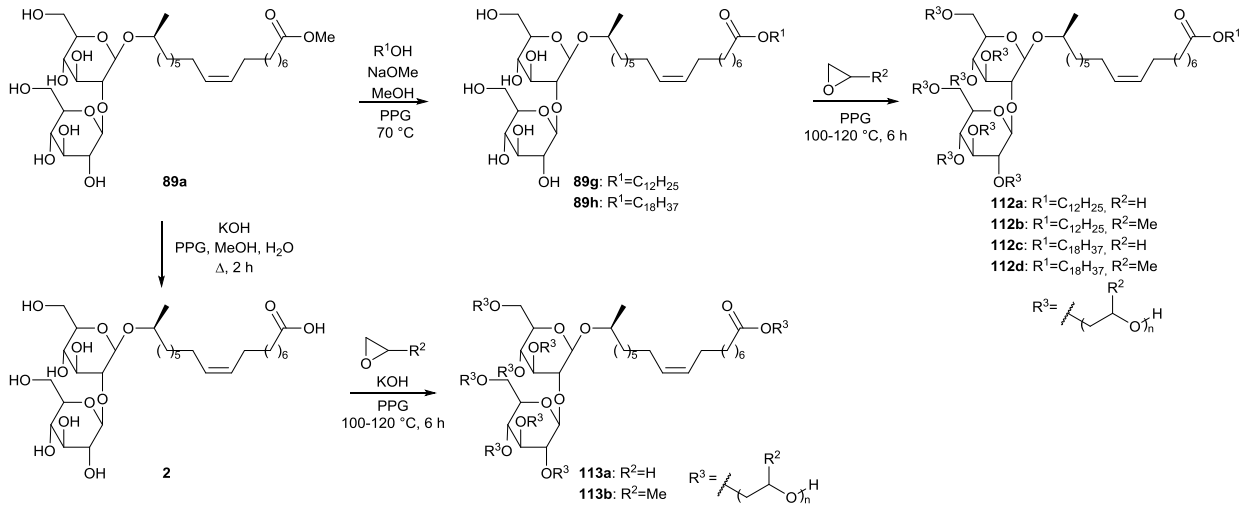
Modifications at the head group of stearic acid based sophorolipids are described by Zerkowski *et al*. (Scheme 17).[81] The crude sophorolipid fermentation product is subjected to alkaline hydrolysis and alcoholysis, yielding the sophorolipid free acid and the sophorolipid ethyl ester **109a**. The sophorolipid free acid is transformed into the sophorolipid benzyl ester **109b** through esterification with benzyl alcohol. Head group modified sophorolipid esters **110** were obtained through coupling of protected amino acids possessing a *para*-aminobenzoic acid linker (Paba) with the carbohydrate hydroxyl groups. The presence of a Paba-linker should avoid decoupling of the amino acids under

alkaline conditions and an excess of sophorolipid ester is used to favor mono-acylation. Two major isomers are obtained for each derivative, most probably the 6' and 6" adducts. Acidolytic or hydrogenolytic deprotection yields the water-soluble sophorolipids **111** with charged head groups. CMC values were determined for the derivatives at different pH-values and were 5.3-27.1 mg/L (6-24 µM) for compounds **111a-e** and 54.1-116.7 mg/L (51-110 µM) for compounds **111f-g**. The results show a significant impact of the modified head groups on the surface tension-lowering properties.

A patent was published by Inoue *et al.* on the synthesis of hydroxyalkyl-etherified sophorolipid esters (Scheme 18).[82] The crude sophorolipid fermentation product was subjected to methanolysis to produce sophorolipid methyl ester **89a**. After transformation towards sophorolipid esters **89g** and **89h** or sophorolipid acid **2**, the hydroxyalkyl-etherified sophorolipid esters **112** and **113** were produced through reaction with alkylene oxides in the presence of an alkali catalyst. These hydroxyalkyl-etherified sophorolipid esters were incorporated in cosmetic compositions to prepare powdered compressed cosmetic materials such as face powder, cheek rouge, highlight or eye shadow or stick-shaped cosmetic materials such as stick-shaped rouge, lip cream, stick-shaped eye shadow or cosmetic pencil.[83-84] They were also applied in cosmetics as moisture-retaining and moisturizing agents, giving a less sticky and better moisturizing feeling than conventional moisturizers such as glycerin, sorbitol and ethylene glycol.[85] The hydroxyalkyl-etherified sophorolipid esters were formulated as moisturizers in hand cream, cleansing milk, skin lotion, facial pack and lip stick.



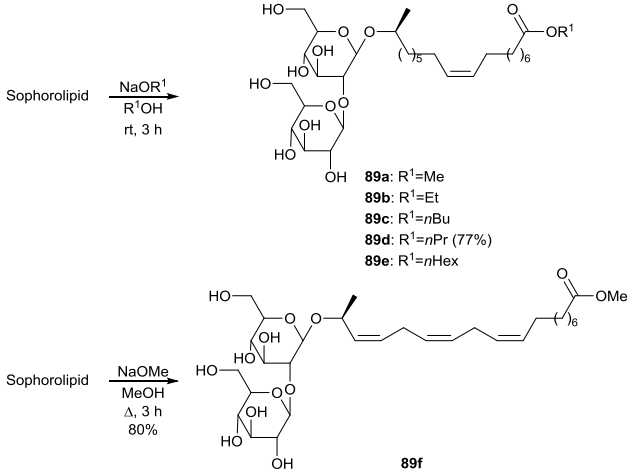
Scheme 17. Modification at the head group with amino acids towards charged sophorolipid derivatives



Scheme 18. Synthesis of hydroxy-alkyl etherified sophorolipids 112 and 113

2.4.3. Modifications at the end of the lipid tail

A first modification at the end of the lipid tail, *i.e.* the synthesis of sophorolipid alkyl esters **89**, was already described in Scheme 11. More sophorolipid alkyl esters **89** were synthesized by Zhang *et al*. in order to evaluate the relationship between the length of the alkyl ester chain and the interfacial properties of the derivatives (Scheme 19).[86] The surface tension was measured for all the derivatives. Critical micelle concentration (CMC) and the minimum surface tension decreased for increasing alkyl ester chain length. Adsorption at solid/liquid interfaces was studied as well, demonstrating adsorption on alumina but much less on silica. Hydrogen bonding was proposed to be the primary driving force for adsorption on alumina and the maximum adsorption density suggested bilayer formation at higher concentrations.

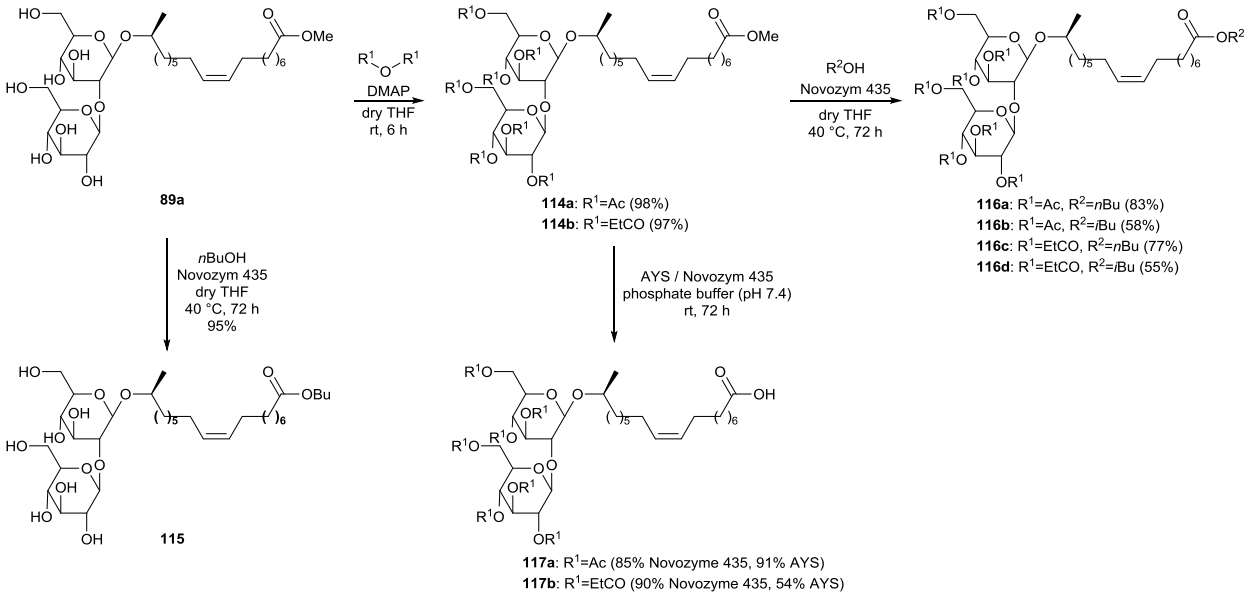


Scheme 19. Alkaline alcoholysis towards sophorolipid esters 89

The transesterification of an α-linolenic acid based sophorolipid mixture into sophorolipid methyl ester **89f** was described by Gupta & Prabhune (Scheme 19).[87] Their minimum inhibitory concentrations against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were

determined and proved to be respectively 20, 10 and 10 µg/mL for the fermentation product and respectively >20, 20 and 20 µg/mL for sophorolipid methyl ester **89f**.[88]

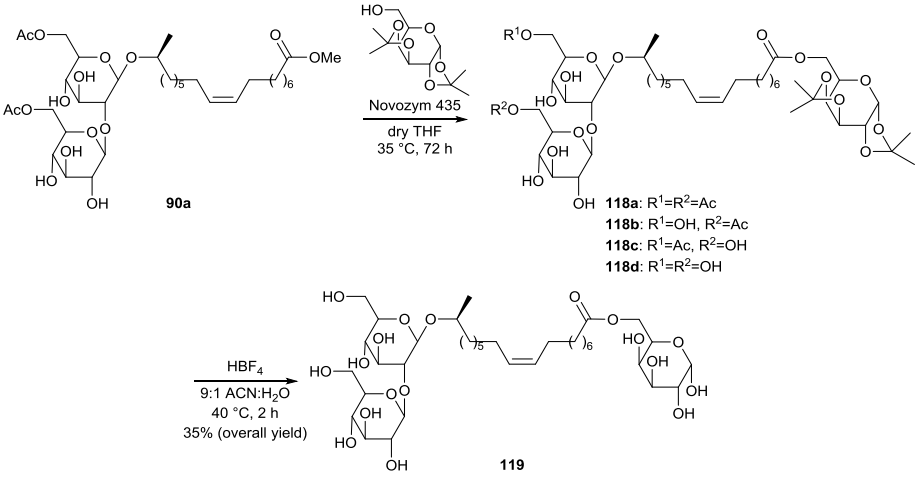
Enzyme-catalyzed transesterifications at the lipid tail are described by Carr & Bisht (Scheme 20).[89] Sophorolipid methyl ester **89a** was transformed into the corresponding peracylated sophorolipid methyl esters **114** through reaction with acetic and propionic anhydride in the presence of a catalytic amount of DMAP. Different enzymes were evaluated for the transesterification in presence of *n*-butanol and *i*-butanol. Only Novozym 435 proved to be successful for the transesterification of both methyl ester **89a** and peracylated methyl esters **114** respectively, into sophorolipid butyl ester **115** and peracylated sophorolipid esters **116**. Deacylation of the sugar head did not occur. It is suggested that the presence of the macrolactonic ring is necessary to fit the sugar head in the binding pocket of the lipase for deacylation to occur. A lipase catalyzed hydrolysis of the lipid tail was performed with AYS and Novozym 435 in a phosphate buffer, yielding peracylated sophorolipid acids **117**.



Scheme 20. Enzyme-catalyzed transesterifications at the lipid tail

Transesterification at the lipid tail was extended by Nuñez *et al*. for the coupling of a sugar monomer to the diacetylated sophorolipid methyl ester **90a** (Scheme 21).[90] A first attempt with glucose was not successful due to its poor solubility in organic solvents. Functionalization of the hydroxyl groups proved necessary where ketalization is favored for its selectivity. Galactose was used instead of glucose since the primary alcohol still has to be available for the transesterification. The transesterification reaction was performed with immobilized Novozym 435 and sophorolipid sugar esters **118** were formed which varied in the degree of acetylation. Deacetylation occurred through the lipase-catalyzed transesterification of the acetylated primary alcohols and the functionalized galactose unit. Deacetylation at the 6”-position resulted in the formation of 1,6”-sophorolipid lactone

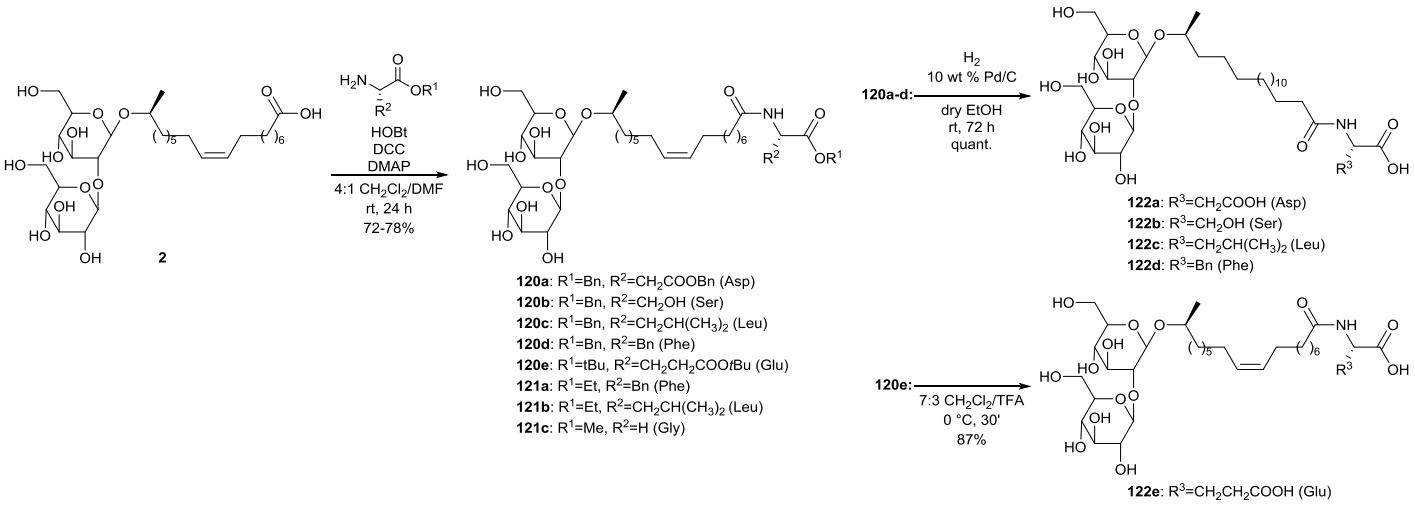
91 (9%) and its 6'-acetylated analogue (14%) (Scheme 11). Acid hydrolysis with HBF_4 was performed for the deprotection of the acetyl and ketal groups towards sophorolipid galactopyranose ester **119**.



Scheme 21. Transesterification towards sophorolipid galactopyranose ester 119

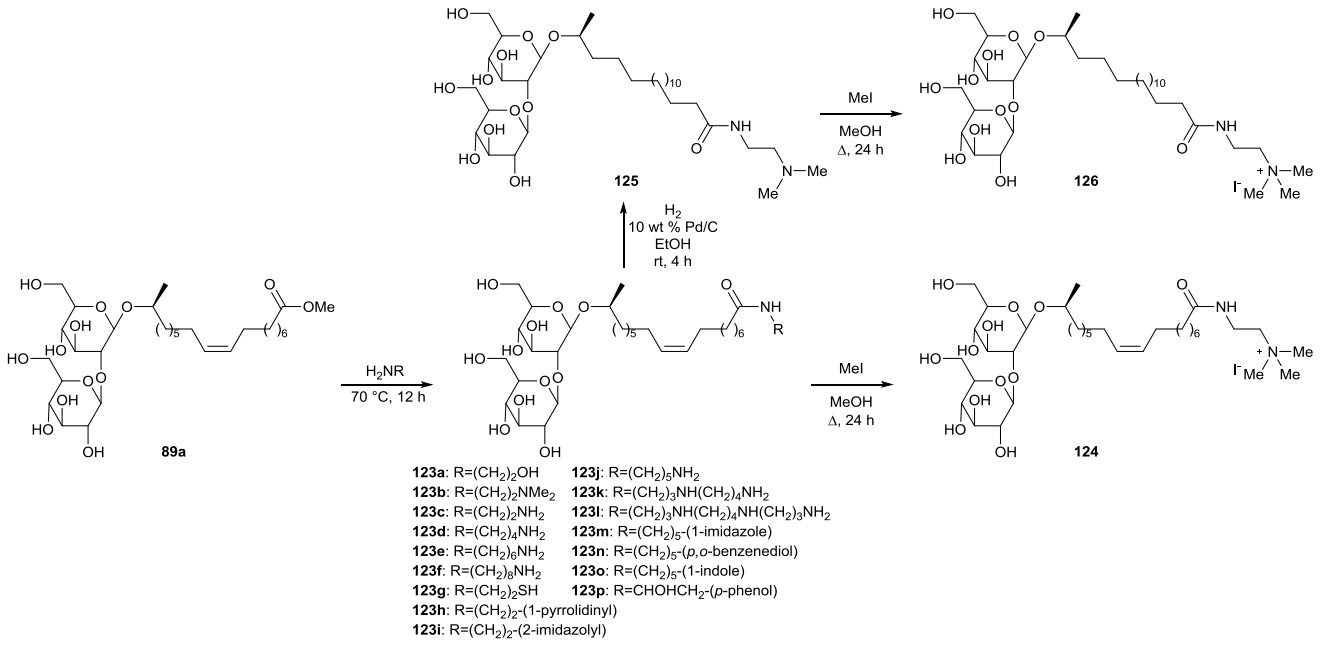
Coupling of sophorolipid acid **2** with different amino acids is described by Azim *et al*. (Scheme 22).[91] The crude fermentation product was transformed into sophorolipid acid **2** through alkaline hydrolysis with NaOH. Protected amino acid conjugated sophorolipids **120** and amino acid conjugated sophorolipid alkyl esters **121** were synthesized using DCC coupling. Deprotection of the different protecting groups yielded amino acid conjugated sophorolipids **122**. Deprotection of benzyl ester groups in compounds **120a-d** was performed by hydrogenation on Pd/C which simultaneously resulted in the reduction of the double bond. Deprotection of *t*-butyl groups in compound **120e** was accomplished under acid conditions.

The antimicrobial, anti-HIV and spermicidal activity were evaluated for amino acid conjugated sophorolipid alkyl esters **121** and amino acid conjugated sophorolipids **122**. All tested derivatives possessed antimicrobial activity against Gram-positive and Gram-negative organisms. The best results were obtained for leucine conjugated sophorolipid **122c**, with MIC values of 1 and 2 µg/mL against respectively *Moraxella* sp. and *S. sanguinis*. Also the leucine conjugated sophorolipid ethyl ester **121b** featured high activity, especially against *Moraxella* sp. with a MIC value of 830 µg/mL. Monoacetylated ethyl ester sophorolipid (MAEE) was also included in the antimicrobial screening and showed higher activity against all the organisms except for leucine conjugated sophorolipid ethyl ester **121b** against *Moraxella* sp. All tested derivatives displayed virus inactivation with an EC_{50} below 200 µg/mL. The esterified derivatives **121** are more potent than the corresponding non-esterified compounds **122**. Leucine conjugated sophorolipid **121c** was the most potent and displayed even higher anti-HIV activity than the commercial spermicide nonoxynol-9 (EC_{50}≈65 µg/ml). None of the tested derivatives displayed significant spermicidal activity. Nonoxynol-9 and MAEE were used as positive controls.



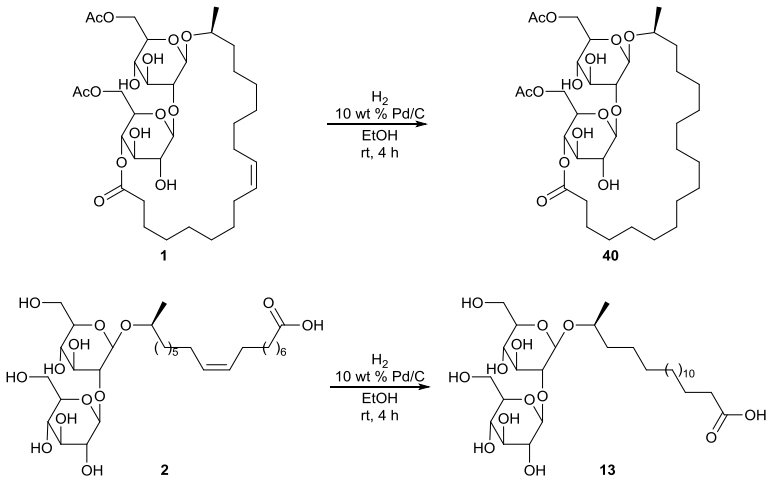
Scheme 22. Synthesis of amino acid conjugated sophorolipids

Synthesis of sophorolipid amides **123** is described in a patent published by Schofield *et al*. (Scheme 23).[92] Non-biogenic sophorolipid amides **123a**-**123i** and biogenic sophorolipid amides **123j**-**123p** were synthesized from sophorolipid methyl ester **89a** through amidation with the corresponding amines. Sophorolipid *N'*,*N'*-dimethylethylamide **123b** was quaternized with methyl iodide to the sophorolipid quaternary ammonium salt **124**. This compound was also hydrogenated to the saturated sophorolipid *N'*,*N'*-dimethylethylamide **125** and subsequently quaternized to the saturated sophorolipid quaternary ammonium salt **126**. Antimicrobial activities were evaluated against a wide variety of micro-organisms.



Scheme 23. Synthesis of non-biogenic and biogenic sophorolipid amides

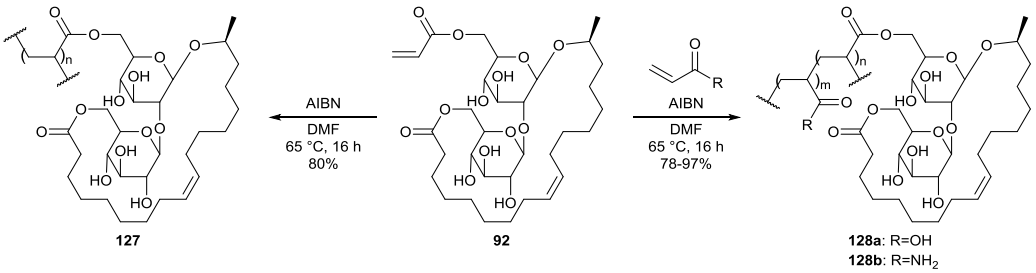
Hydrogenation of diacetylated sophorolipid lactone **1** and sophorolipid acid **2** was also performed, yielding respectively the saturated diacetylated sophorolipid lactone **40** and the saturated sophorolipid acid **13** (Scheme 24).[92] Antimicrobial activities were evaluated against a wide variety of micro-organisms.



Scheme 24. Hydrogenation of diacetylated sophorolipid lactone 1 and sophorolipid acid 2

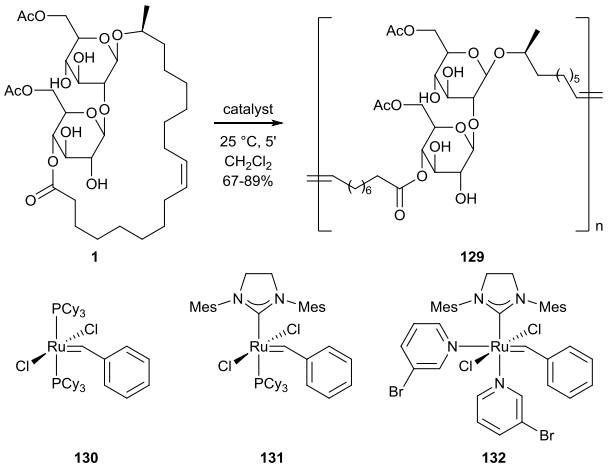
2.4.4. Sophorolipid polymerization

The sophorolipid lactone **92** was used by Bisht *et al*. for the synthesis of polymers with amphiphilic properties (Scheme 25).[93] Homopolymerisation was performed with AIBN as initiator, yielding sophorolipid homopolymer **127**. This polymer is soluble in DMF and DMSO but not in water. To increase the hydrophilic character of the polymer, copolymers **128** were produced with acrylic acid and acrylamide. The copolymer composition was controlled by the monomer feed ratio. Copolymers **128a** and **128b** were synthesized with respectively 3, 5, 10 and 50 mol % and 0.5, 1, 2, 3, 5 and 50 mol % sophorolipid lactone **92** in the feed. Copolymer **128b** is soluble in water with less than 3 mol % sophorolipid lactone **92** in the feed.



Scheme 25. Homo- and copolymerization of sophorolipid lactone 92

A ring-opening metathesis polymerization (ROMP) for the synthesis of a poly(sophorolipid) **129** from sophorolipid lactone **1** was first described by Gao *et al*. (Scheme 26).[94] The presence of degradable links in the polymer structure makes these polymers promising bioresorbable materials. Three different ruthenium-based Grubbs catalysts were evaluated and the highest yields were obtained with catalyst **131**.

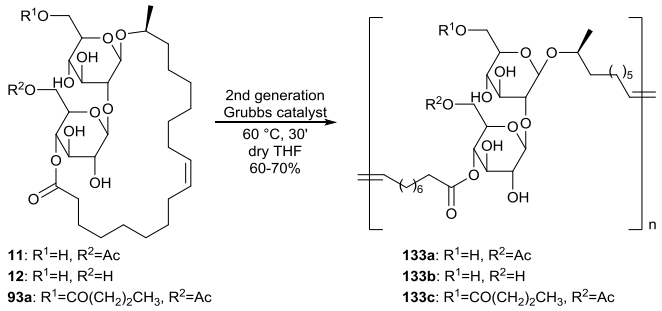


Scheme 26. Ring-opening metathesis polymerization towards poly(sophorolipid) 129

The solid-state properties of this poly(sophorolipid) were evaluated by Zini *et al*.[95] Both *Z* and *E* double bonds are present in the poly(sophorolipid), respectively in 90% and 10%. Thermogravimetric analysis demonstrated the presence of two main degradation steps. The first step around 350 °C may be associated with the degradation of the hydrophilic sugar moieties, the second step around 450 °C can be attributed to the thermal degradation of the hydrophobic fatty acid chain. The combined results of differential scanning calorimetry (DSC), X-ray diffraction (XRD) and temperature modulated DSC (TMDSC) suggest that the poly(sophorolipid) possesses crystalline domains which melt at 123 °C and has a glass transition at 61 °C. A kinetic study on the polymerization mechanism was performed by Peng *et al*., categorizing it as an enthalpy driven ROMP.[96] The effect of catalyst loading, monomer concentration and solvent choice were evaluated for polymerization with Grubbs catalysts **131** and **132**.

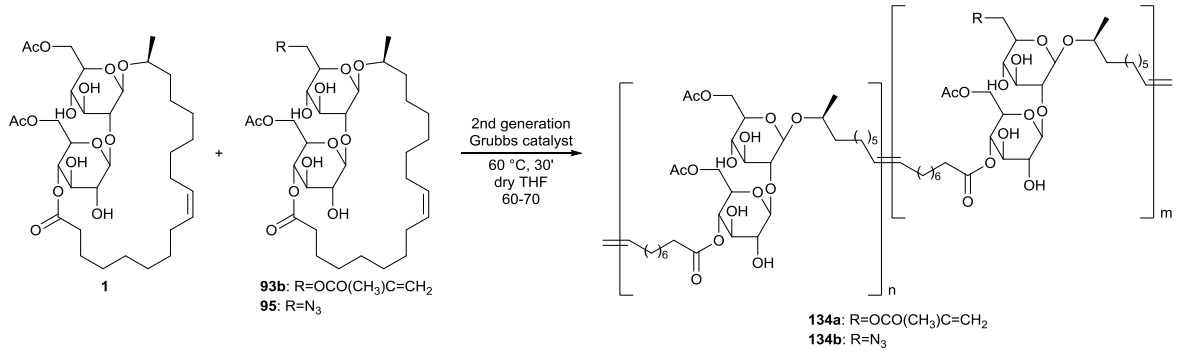
The structural diversity of the poly(sophorolipid) was expanded by Peng *et al*. through the incorporation of modified sophorolipid lactone monomers in the polymer product (Scheme 27).[75] The di-acetylated, mono-acetylated, non-acetylated and butyrated sophorolipid lactones **1**, **11**, **12** and **93a** were homopolymerized according the optimal conditions which were determined in the kinetic study. TGA analysis showed that all polymers are stable up to 200°C and DSC analysis was used to analyze their thermal behavior. No melting transitions were observed for the mono- and non-acetylated sophorolipid polymers, probably due to the absence of a crystalline phase caused by a restricted chain mobility. These polymers show significant potential as substrates for bone tissue engineering due to their solid-state properties. Biological properties relevant for tissue engineering were determined for all polymers except for the non-acetylated sophorolipid polymer **133b** due to stability problems. Cytotoxicity on human mesenchymal stem cells was evaluated *via* LDH and cell proliferation assays and a similar cytocompatibility was observed as the control tissue culture polystyrene (TCPS). All three polymers also displayed similar cell adhesion and spreading as the

control CTPS. The capacity of the three polymers to support the differentiation of the mesenchymal stem cells to an osteoblast cell phenotype was evaluated, giving the best results for butyrated sophorolipid polymer **133c** compared to the control TCPS. Finally, degradation of the polymers in an osteogenesis medium was determined. All three polymers underwent an appreciable molecular weight loss in fourteen days and the degradation rate was dependent on the substitution at the sugar moiety.



Scheme 27. Homopolymerization of sophorolipid lactone derivatives

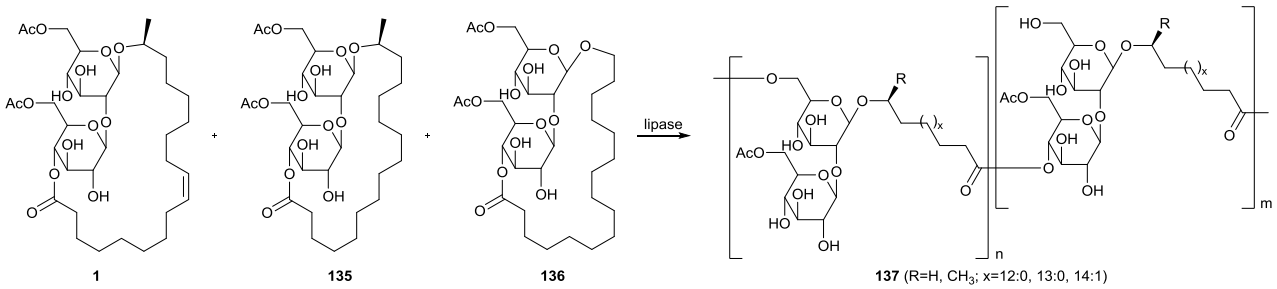
Copolymers of diacetylated sophorolipid lactone **1** and methacrylated or azidated sophorolipid compounds were prepared with control of the presence of the clickable functional groups (Scheme 28).[75] Functionalization of the methacrylate containing copolymer **134a** *via* a thiol-ene reaction was performed with mercaptoethanol as a model compound. Similarly, functionalization of the azide containing copolymer **134b** was performed with 3-butyn-1-ol *via* the azide-alkyne cycloaddition reaction. Copolymers containing both methacrylate and azide groups were also prepared.



Scheme 28. Synthesis of copolymers with methacrylate and azide groups

Ring-opening polymerization of sophorolipid lactones with lipases was explored by Hu & Ju (Scheme 29).[97] The starting material for the polymerization was a mixture of ω-1 C18:1 diacetylated sophorolipid **1**, ω-1 C16:0 diacetylated sophorolipid **135** and ω C16:0 diacetylated sophorolipid **136**. Ethyl acetate, pyridine, isopropyl ether, toluene, cyclohexane and hexane were evaluated as solvents for polymerization with porcine pancreatic lipase (PPL), giving the highest conversion in isopropyl ether and toluene. An evaluation of the reaction mechanism revealed that the diacetylated sophorolipid lactones were selectively deacetylated at the 6'-position prior to the ring-opening

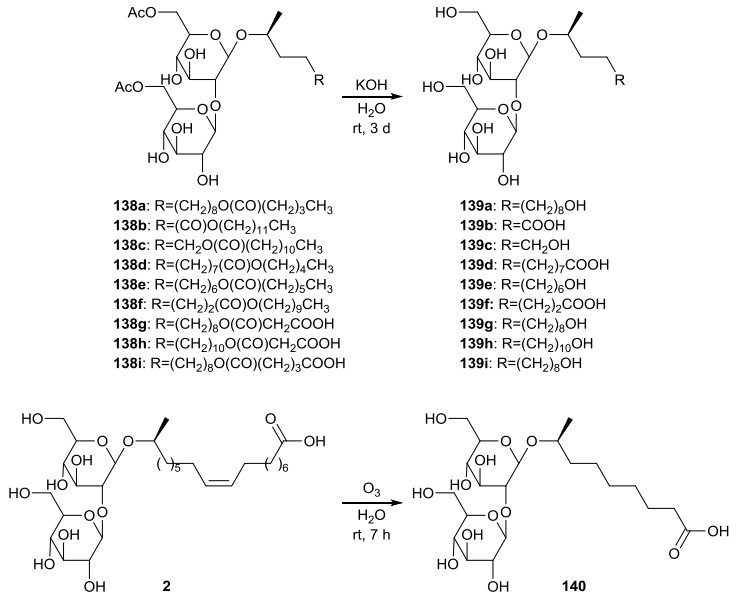
polymerization. This results in linkage of the monomers *via* both the 6'-position and the 4"-position at the sugar moiety. Four different lipases were used to evaluate the conversion efficiency in both acetonitrile and isopropyl ether, namely PPL, immobilized *Mucor miehei* lipase (MML), lyophilized *Candida antarctica* lipase (CAL-B) and lyophilized *Pseudomonas* sp. lipase (PSL). The highest conversion was obtained with MML in isopropyl ether, but CAL-B proved to be the least solvent sensitive. Substrate selectivity depended on the temperature, with an increasing conversion at 60 °C but decreasing conversion at 50 °C for larger ring sizes. The overall polymerization proceeded the fastest at 60 °C.



Scheme 29. Enzymatic ring-opening polymerization of sophorolipid lactones

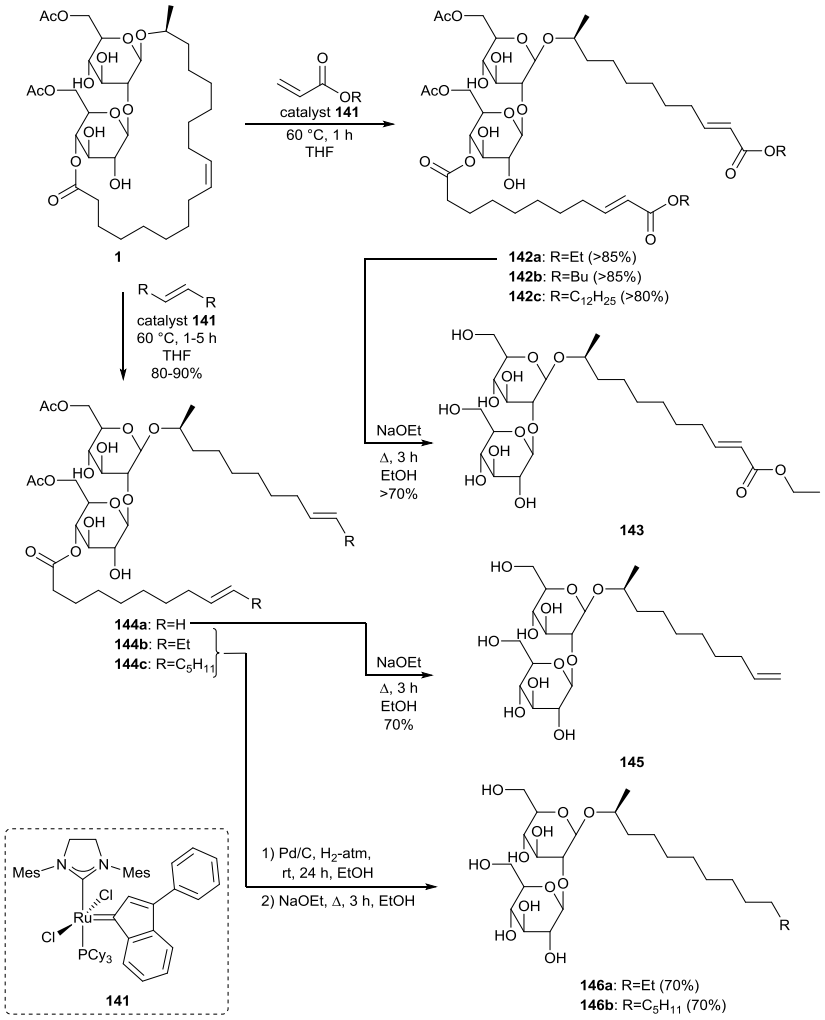
2.4.5. Modifications towards short-chained sophorolipids

The synthesis of short-chained sophorolipids was described by Develter and Fleurackers, and by Van Bogaert *et al*. (Scheme 30).[26, 98] The esters dodecyl pentanoate, pentyl dodecanoate, decyl heptanoate, dodecyl malonate, myristyl malonate and dodecyl glutarate were used for the synthesis of sophorolipid esters **138** by *Starmerella bombicola*. These sophorolipid esters were subjected to alkaline hydrolysis, yielding short-chained sophorolipids **139**. An alternative strategy for the production of short-chained derivatives implied the ozonolysis reaction of sophorolipid acid **2** in water towards C9 sophorolipid acid **140**. The mixture of this short-chained sophorolipid acid and azelaic acid reduced the surface tension of water to 30 mN/m and dynamic surface tension measurements indicated that the reduction of the surface tension was faster compared to crude sophorolipids after fermentation. The mixture proved to be a better wetting agent than Simulsol AS48 and a comparable hydrotrope as Simulsol AS48 and crude sophorolipids.



Scheme 30. Synthesis of short-chained sophorolipids

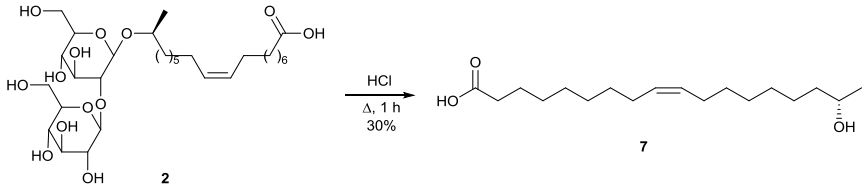
Ring-opening cross-metathesis of sophorolipid lactone **1** was performed by Peng *et al*. for the synthesis of short-chained sophorolipid derivatives (Scheme 31).[99] The cross-metathesis reactions with *n*-acrylates, trans-3-hexene, 1-hexene and ethylene were catalyzed with a second generation Grubbs catalyst **141**. Cross-metathesis with *n*-acrylates resulted in the synthesis of three gemini type sophorolipids **142**. Subsequent ethanolysis reaction yielded the corresponding ethyl ester **143**. Cross-methathesis with trans-3-hexene, 1-hexene and ethylene followed by ethanolysis and optional hydrogenation yielded short-chain alkyl sophorolipids **145** and **146**. Hydrogenation of unsaturated alkyl sophorolipid **145** was not performed since this terminal double bond is valuable for further functionalization. Critical micelle concentrations were determined *via* the Wilhelmy plate method and *n*-dodecyl-β-*D*-maltoside was used as a reference compound for comparison. Both the CMC and minimum surface tension (γ_{min}) decrease with increasing hydrophobicity and are thus dependent on the chain length. The maximum surface adsorption density (Γ_m) and minimum surface coverage area per surfactant (A_{min}) were also calculated. Increasing the chain length results in a higher packing density and lower minimum surface area, especially from alkyl sophorolipid **145** to alkyl sophorolipid **146a**. Sophorolipid ethyl ester **143** displayed the highest packing density and lowest minimum surface area despite its hydrophilic character. It is thought that the ester moiety induces additional lateral attraction which reduces the distance between the molecules at the air-water interface. Comparison of the values for *n*-dodecyl-β-*D*-maltoside and alkyl sophorolipid **146a** demonstrates that sophorose occupies a larger effective area than maltose at the air-water interface. The influence of the chain length of alkyl sophorolipids on the CMC proved to be smaller than that of alkyl glucoside and maltoside sugar-based surfactants. This is likely due to the higher effective area occupied by the sophorose group.



Scheme 31. Ring-opening cross metathesis towards short-chain sophorolipids

2.4.6. Degradation of sophorolipids in smaller building blocks

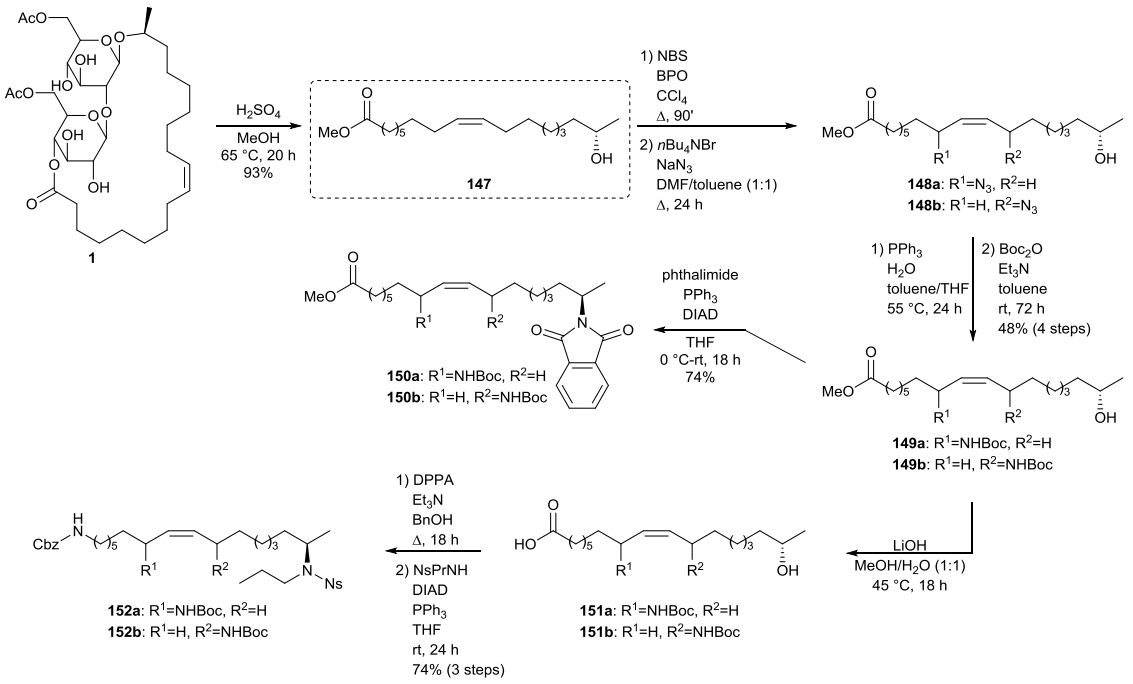
Deacetylated sophorolipid acid **2** was converted into the ω-1 hydroxy fatty acid **7** by Rau *et al*. *via* acid hydrolysis (Scheme 32).[79] The ω-1 hydroxy fatty acid **7** is a rare fatty acid which is difficult to prepare *via* organic synthesis. This fatty acid could be used as a building block for the synthesis of pharmaceutically valuable products and for polyester- and macrocyclic lactones.



Scheme 32. Degradation towards 17-hydroxy oleic acid 7

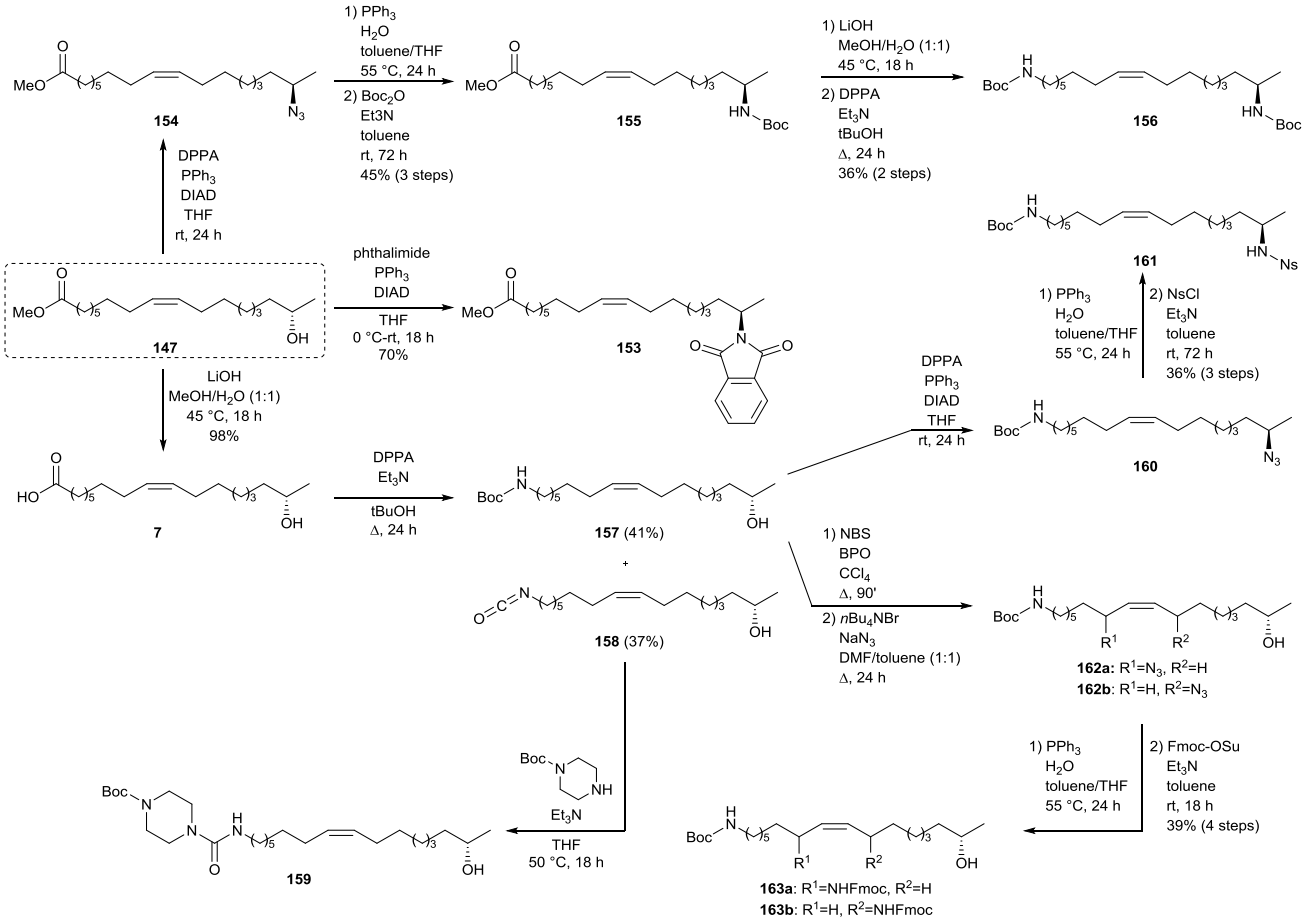
Zerkowski and Solaiman described the synthesis of polyfunctional fatty amines starting from sophorolipids (Scheme 33).[100] In a first step, sophorolipid lactone **1** is subjected to acid methanolysis to yield methyl 17-hydroxy oleate **147**. An allylic substitution was used to introduce an amine function at the allylic position of the double bond. Methyl 17-hydroxy oleate **147** was reacted with

N-bromosuccinimide and sodium azide towards azide **148** and was subsequently subjected to a Staudinger reaction. Protection of the amine function towards Boc-protected amine **149** was performed without intermediate isolation. Amine **149** was further subjected to a Mitsunobu reaction with phthalimide to yield diamine **150**. After conversion of the ester function of amine **149** into a carboxylic acid function, a Curtius rearrangement with diphenylphosphorylazide and a Mitsunobu reaction were performed to yield triamine **152**.



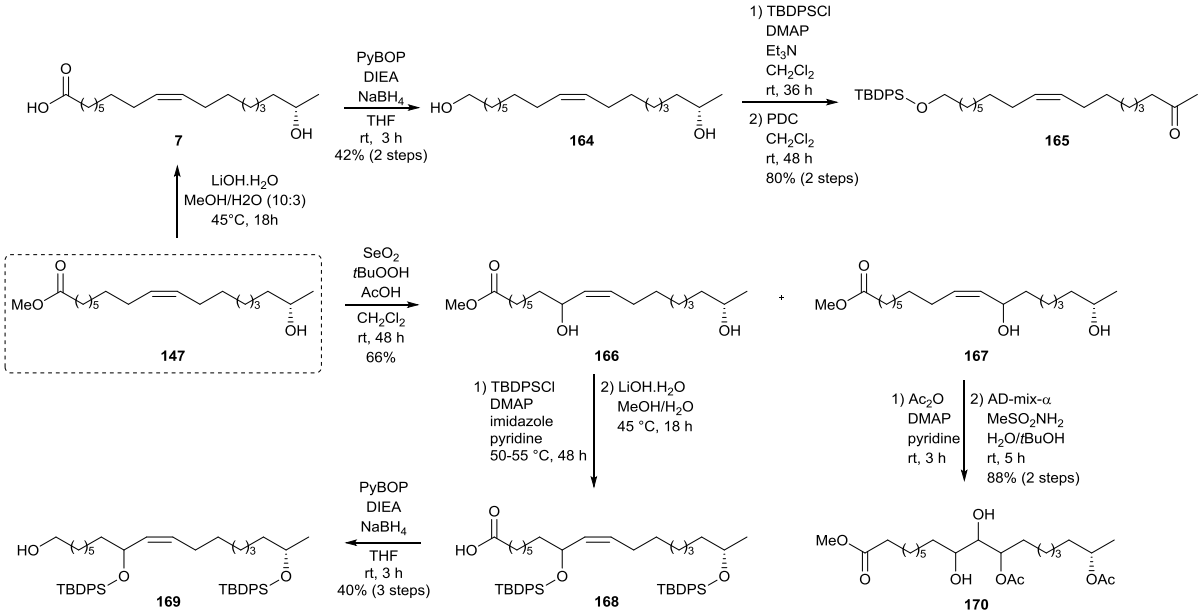
Scheme 33. Synthesis of polyfunctional fatty amines from sophorolipids (part 1)

Further modifications of methyl 17-hydroxy oleate **147** comprised a Mitsunobu reaction towards subterminal amine **153** with phthalimide and the synthesis of azide **154** followed by a Staudinger reaction and protection of the amine function towards Boc-protected amine **155** (Scheme 34). Conversion of the ester function into a carboxylic acid function and a subsequent Curtius rearrangement yielded diamine **156**. Finally, methyl 17-hydroxy oleate **147** was converted into 17-hydroxy oleic acid **7** and was subsequently subjected to a Curtius rearrangement to yield amine **157** and isocyanate **158**. The latter was converted into urea derivative **159** with mono-Boc piperazine. Amine **157** was transformed into azide **160** and subsequently subjected to a Staudinger reaction with protection of the amine function towards Ns-protected amine **161**. Amine **157** was also reacted with *N*-bromosuccinimide and sodium azide towards azide **162** and additionally subjected to a Staudinger reaction and protection of the amine function towards Fmoc-protected amine **163**.



Scheme 34. Synthesis of polyfunctional fatty amines from sophorolipids (part 2)

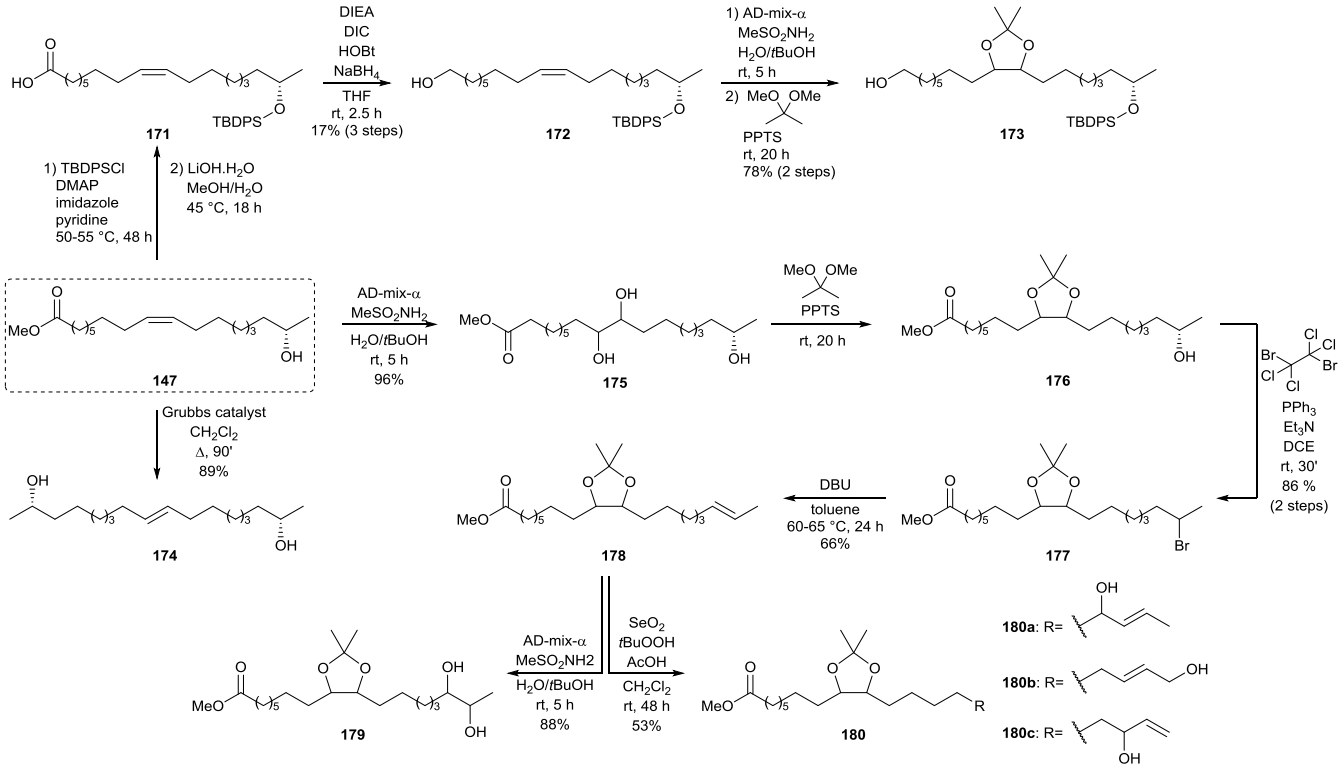
A similar strategy was adopted by Zerkowski and Solaiman for the synthesis of polyhydroxy fatty acids (Scheme 35).[101] Methyl 17-hydroxy oleate **147** was converted into 17-hydroxy oleic acid **7** and subsequently reduced towards diol **164** *via* an intermediate activated ester. The primary alcohol was selectively protected with *t*-butyldiphenylsilyl chloride after which the secondary alcohol was oxidized with pyridinium dichromate towards keton **165**. Allylic hydroxylation with selenium dioxide of methyl 17-hydroxy oleate **147** yielded two isomers **166** and **167**. The former was protected with *t*-butyldiphenylsilyl chloride, converted to carboxylic acid **168** and reduced to the primary alcohol **169**. The latter was protected with acetic anhydride and subjected to the Sharpless' asymmetric dihydroxylation towards methyl ester **170**.



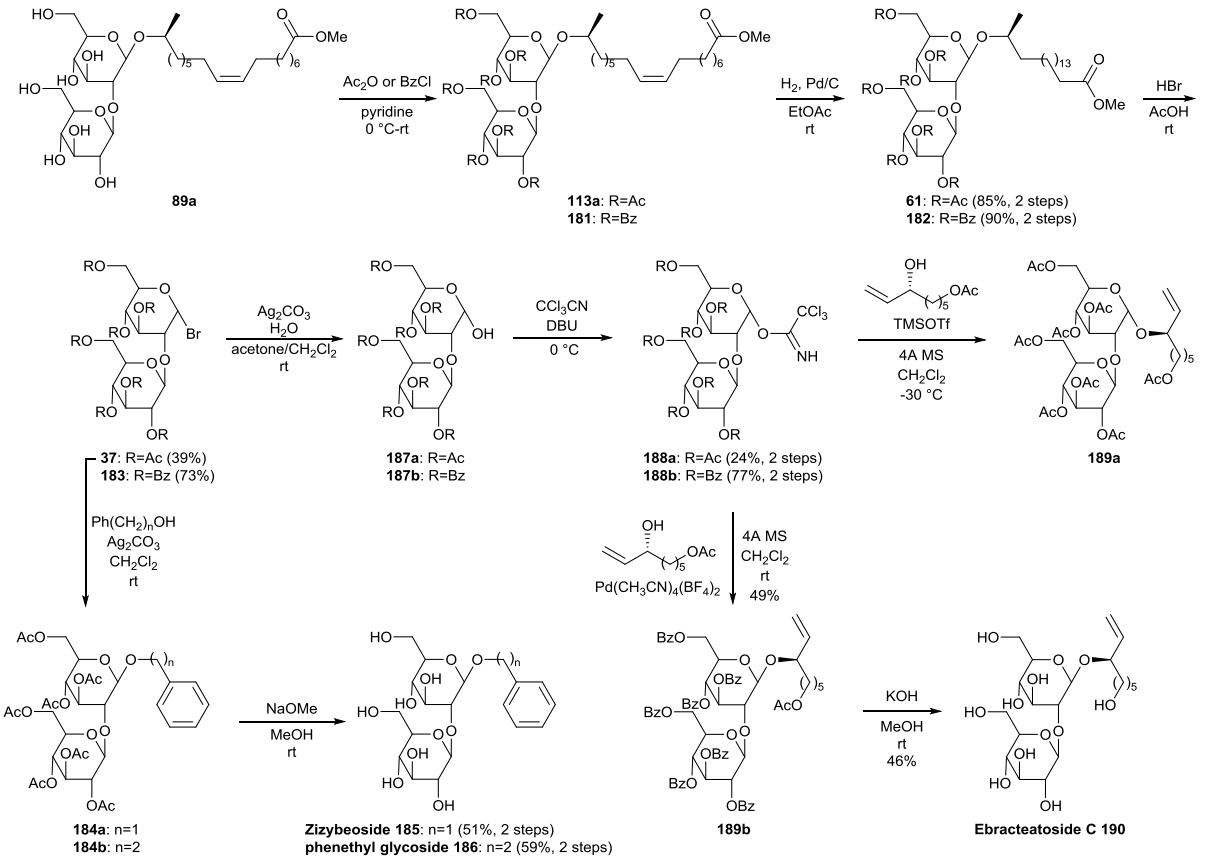
Scheme 35. Synthesis of polyhydroxy compounds from sophorolipids (part 1)

Protection of methyl 17-hydroxy oleate **147** with *t*-butyldiphenylsilyl chloride followed by hydrolysis yielded carboxylic acid **171**, which was subsequently reduced to alcohol **172** (Scheme 36). Sharpless' asymmetric dihydroxylation and protection with 2,2-dimethoxypropane yielded alcohol **173**. Olefin metathesis of methyl 17-hydroxy oleate **147** with a Grubbs' first generation catalyst resulted in diol **174**. Methyl 17-hydroxy oleate **147** was also directly subjected to Sharpless' asymmetric dihydroxylation towards methyl ester **175** and subsequently protected with 2,2-dimethoxypropane towards methyl ester **176**. Treatment with 1,2-dibromotetrachloroethane resulted in bromide **177**. A new double bond was formed upon elimination with DBU towards methyl ester **178**. Sharpless' asymmetric dihydroxylation yielded methyl ester **179** whereas allylic hydroxylation with selenium dioxide resulted in three isomeric products **180**.

Sophorolipids were applied by Hoffmann *et al*. in the synthesis of the natural products Ebracteatoside C, Zizybeoside I and phenethyl glucoside (Scheme 37).[102] The crude sophorolipid fermentation product was transformed into the sophorolipid methyl ester **89a**. After protection of the carbohydrate hydroxyl groups and reduction of the double bond, sophorolipid methyl esters **61** and **182** were transformed into anomeric sophorose bromides **37** and **183**. The acetylated sophorose bromide **37** was then treated under Koenigs-Knorr conditions with benzyl alcohol or 2-phenethylethanol to yield Zizybeoside **185** or phenethyl glycoside **186** after deprotection under Zemplén conditions. Sophorose bromides **37** and **183** were hydrolyzed and subsequently reacted with trichloroacetonitrile to form the trichloroacetimidates **188**. Glycosylation of the acetate protected derivative **188a** selectively led to α-anomer **189a**. Benzoate protected derivative **188b** was glycosylated with $Pd(CH_3CN)_4(BF_4)_2$ to selectively form the enriched β-anomer **189b** and was subsequently hydrolyzed to furnish Ebracteatoside C **190**.



Scheme 36. Synthesis of polyhydroxy compounds from sophorolipids (part 2)



Scheme 37. Synthesis of natural products from sophorolipids

2.5. Sophorolipid based nanoparticles

The application of sophorolipids for the formation of magnetic cobalt nanoparticles is described by Kasture *et al*. (Figure 3, Scheme 38).[103] Sophorolipids have the advantage over oleic acid that they are better water soluble which resulted in the formation of more stable nanoparticles. The cobalt nanoparticles were synthesized by reduction of Co^{2+} with sodium borohydride using the sophorolipid acid **2** as capping agent. The nanoparticles were obtained from aqueous dispersions as a stable powder *via* simple centrifugation or magnetic separation and could easily be redispersed in water. Transmission electron microscopy (TEM) revealed that well separated, polydisperse particles were obtained with an average particle size of around 50 nm. The binding of the sophorolipids to the cobalt nanoparticle surface *via* the carboxylic acid function and the double bond was confirmed by Fourier transform infrared spectroscopy (FTIR). Room-temperature magnetization measurements were performed to demonstrate the good magnetic features of the sophorolipid-capped nanoparticles.

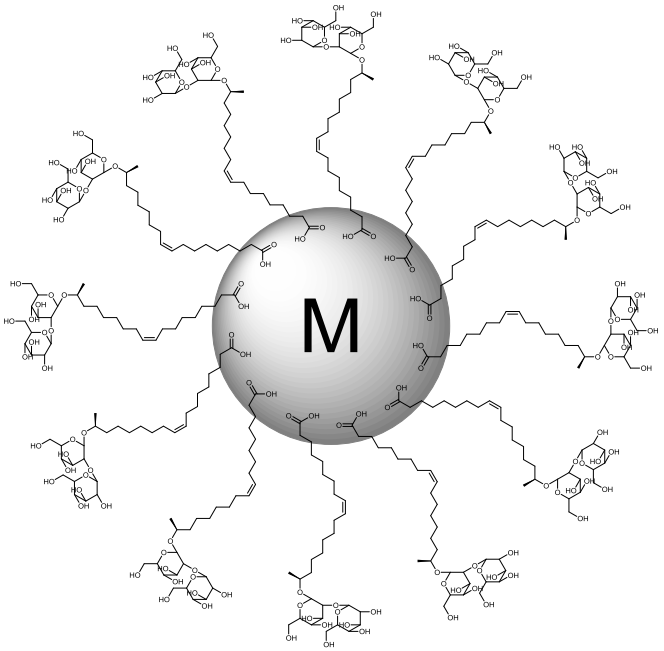
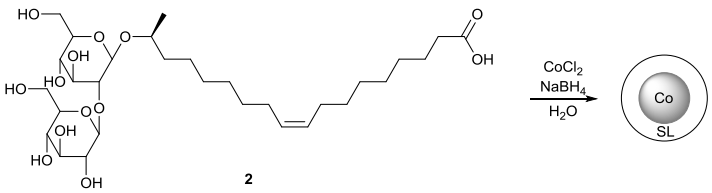


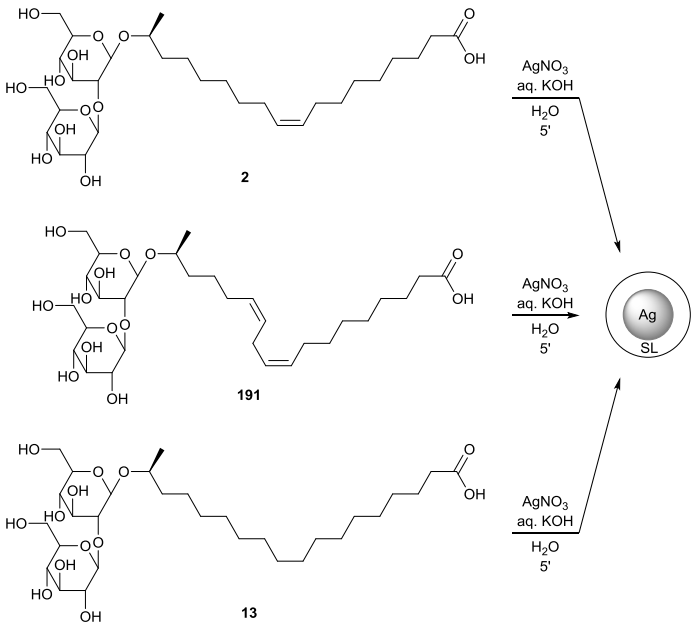
Figure 3. Sophorolipids as nanoparticle capping agents



Scheme 38. Sophorolipid-capped cobalt nanoparticles

Kasture *et al*. also described the formation of sophorolipid based silver nanoparticles (Scheme 39).[104] The sophorolipid acids **2** and **191** were used as reducing and capping agents in the synthesis of the nanoparticles with silver nitrate under alkaline conditions at different temperatures. The differences

in size and dispersion for oleic acid sophorolipid-capped nanoparticles (OA) and linoleic acid sophorolipid-capped nanoparticles (LA) at different temperatures were evaluated. Particle size distribution was determined *via* both transmission electron microscopy (TEM) and dynamic light scattering measurements (DLS). It was observed that particle size decreased with increasing temperature and longer time is needed at low temperatures to obtain complete particle formation and growth.

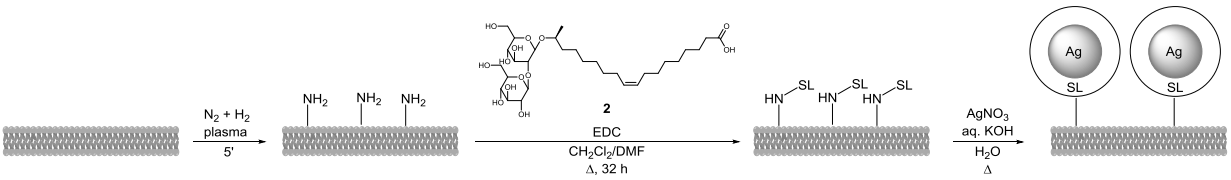


Scheme 39. Sophorolipid-capped silver nanoparticles

The nanoparticles were isolated as a stable powder *via* centrifugation and air-drying which could easily be redispersed in water.[105] Just like for the cobalt nanoparticles, binding of the sophorolipids to the silver nanoparticle surface occurs *via* the carboxylic acid function and the double bond. Continuous flow synthesis of sophorolipid-capped silver nanoparticles is described by Kumar *et al*.[106] Stearic acid based sophorolipid **13** was used as reducing and capping agent for the continuous flow experiments since the resulting nanoparticles were formed faster and featured a better size uniformity than those synthesized with oleic acid based sophorolipid **2**. The influence of the flow rate on the average particle size and particle size distribution were evaluated. Higher flow rates led to better mixing but reduced residence time, which resulted in an incomplete reaction towards large polydisperse particles. At lower flow rates, the reaction was complete and small monodisperse spherical nanoparticles were obtained. The synthesis of sophorolipid-capped silver nanoparticles *via* segmented flow in a microreactor was performed by Kumar *et al*.[107] Both liquid-liquid and gas-liquid segmented flows were applied with respectively kerosene and air as inert phase. The particle size was much smaller for gas-liquid flow than for liquid-liquid flow and the particle size distribution could be controlled by the choice of inert phase. Singh *et al*. investigated the antibacterial activities of these sophorolipid-capped silver nanoparticles.[105] For sophorolipids as such, good inhibition was

obtained against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* while there was only a faint inhibition against the Gram-negative bacterium *Pseudomonas aeruginosa*. In case of the sophorolipid-capped nanoparticles, *Bacillus subtilis* and *Staphylococcus aureus* were exposed to concentrations ranging from 20 to 100 µg/mL. The cell survival of *Bacillus subtilis* dropped to 0.4% after one hour, but no survival was observed for *Pseudomonas aeruginosa*. Sophorolipid-capped silver nanoparticles are thus more effective against Gram-negative bacteria. Silver proved to be the cause of the observed antibacterial effects *via* the induction of pore formation in the bacterial cell membrane through the formation of reactive oxygen species.

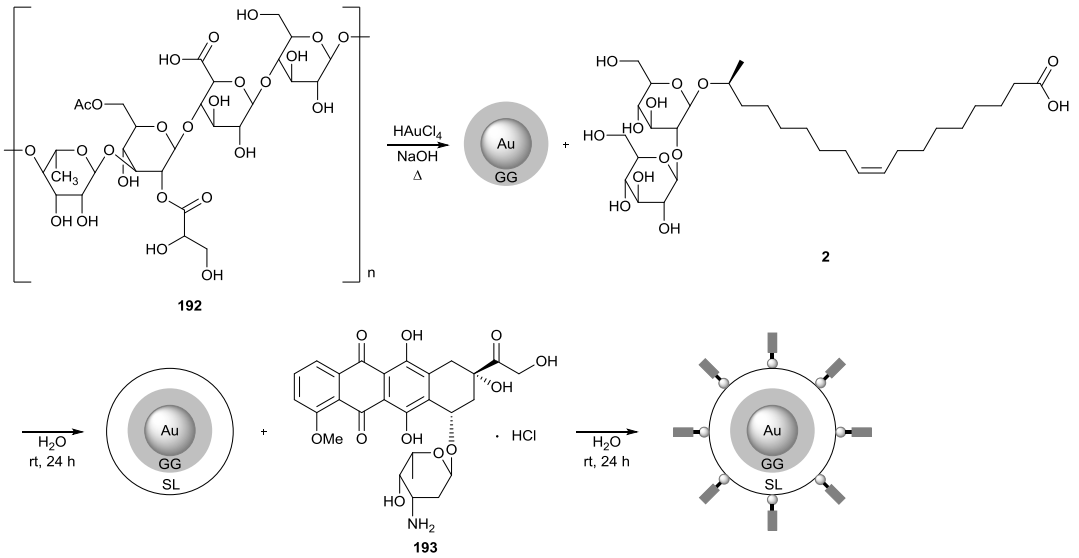
The same methodology was applied by D'Britto *et al*. for the synthesis of silver nanoparticle studded polyethylene scaffolds (Scheme 40).[108] The polymer scaffold was first treated with *in situ* generated ammonia plasma to introduce amine groups. Sophorolipid **2** was covalently attached to the polymer surface *via* an amidation reaction followed by formation of silver nanoparticles at the polymer surface with silver nitrate. The size of these silver nanoparticles was approximately 60 to 70 nm. The antimicrobial activity of the modified polyethylene scaffolds against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* was evaluated. Within six hours of exposure, the silver nanoparticle studded polyethylene scaffolds displayed a broad activity against both the Gram-positive and Gram-negative bacteria. The adhesion and proliferation of mammalian cells on the polymer scaffolds was also evaluated. This revealed that the growth of the mammalian cells was encouraged by the silver nanoparticle studded polyethylene scaffolds and that the cells exhibited good viability. These modified polymer scaffolds are good candidates for tissue-engineering and bio-implant applications.



Scheme 40. Synthesis of silver nanoparticle studded polyethylene scaffolds

Dhar *et al*. described the use of sophorolipids for the synthesis of biocompatible gold nanoparticles (Scheme 41).[109] Gellan gum **192** was used as the primary reducing and capping agent in the formation of the gold nanoparticles with chloroauric acid, followed by conjugation of the nanoparticles with sophorolipid acid **2**. The nanoparticles were further loaded with the anticancer drug doxorubicin hydrochloride **193**. The average particle size was determined *via* transmission electron microscopy and amounted to 13 and 17 nm for respectively the gellan gum capped nanoparticles and the sophorolipid conjugated nanoparticles. After fluorescent labeling, the efficient cellular uptake of the nanoparticles by human glioma cell line LN-229 was demonstrated. The *in vitro*

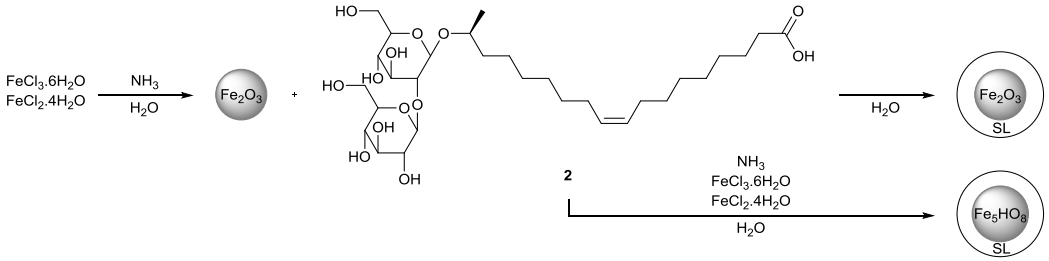
cytotoxicity of both sophorolipid-conjugated nanoparticles and doxorubicin chloride loaded nanoparticles were evaluated and compared to free doxorubicin chloride **193** against human glioma cell line LN-229 and human glioma stem cell line HNHC-2. The doxorubicin loaded nanoparticles performed better then both the sophorolipid-conjugated nanoparticles and the free doxorubicin chloride **193** against both cell lines. This synergistic effect could be explained by the better cell penetration of the doxorubicin loaded nanoparticles compared to free doxorubicin chloride **193**.



Scheme 41. Sophorolipid-conjugated gellan gum capped gold nanoparticles

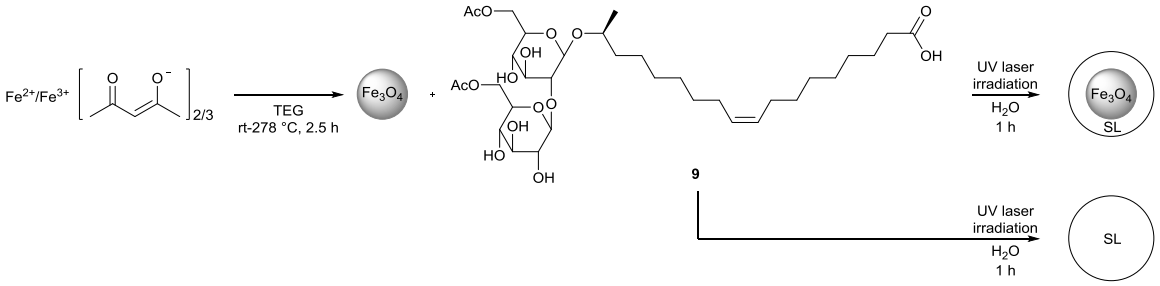
The formation of sophorolipid-capped iron oxide nanoparticles was executed by Baccile *et al*. (Scheme 42).[110] The magnetic iron nanoparticles were synthesized with iron (II) chloride and iron (III) chloride in the presence of ammonia at room temperature and 80°C. Both a two-step and one-step procedure were applied which resulted in nanoparticles with respectively a maghemite (γ-Fe_2O_3) or two-line ferrihydrite ($Fe_5HO_8{\cdot}4H_2O$) structure. Particle size distribution was determined *via* both transmission electron microscopy (TEM) and dynamic light scattering measurements (DLS). A very broad particle size distribution was obtained for the one-way synthesis pathway at room temperature *via* TEM, while monodisperse nanoparticles were obtained in all other cases. A narrow size distribution was obtained for all samples *via* DLS measurement in water and all average sizes are systematically higher than those measured by TEM. When DLS measurements were performed in an ethanol/water mixture (8:2), particle sizes increased compared to those measured in water, indicating the aggregation of the nanoparticles into large aggregates. The much higher increase of the average particle size for the nanoparticles synthesized at room temperature indicated that sophorolipids were more loosely bonded to the nanoparticle surface. FTIR analysis revealed that the sophorolipids are mainly attached to the nanoparticle surface *via* the carboxylic acid function. Adsorption studies were performed for the sophorolipid-capped iron oxide nanoparticles with two

lectines, namely Concanavalin A and lectin from *Bandeiraea simplicifolia*. No specific interaction occurred with the lectin from *Bandeiraea simplicifolia*, but affinity for Concanavalin A was observed. Sophorolipid-capped iron oxide nanoparticles thus proved to be interesting as selective targets for sugar/protein interactions.



Scheme 42. Sophorolipid-capped iron oxide nanoparticles

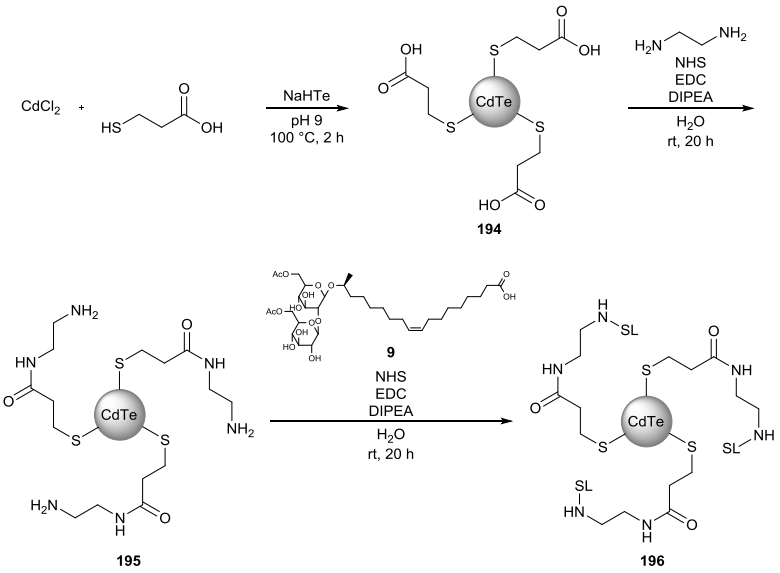
Singh *et al*. described the synthesis of sophorolipid self-assembled vesicular mesostructures *via* laser irradiation and their subsequent loading with iron oxide nanoparticles (Scheme 43).[111] Laser irradiated sophorolipids proved to be highly fluorescent and in the case of diacetylated sophorolipid acid **9**, uniform spherical microstructures were obtained. Upon irradiation, sheet-like structures are formed initially which are converted into fully developed spherical mesostructures after one hour. By adjusting the irradiation conditions, nanoparticles of approximately 100 nm could be obtained. After drying, the sophorolipid mesostructures could easily be redispersed in water. When Fe_3O_4 nanoparticles were added during the synthesis, sophorolipid mesostructures embedded with iron oxide nanoparticles were obtained. An MTT-assay on a HeLa derived cell line revealed that the sophorolipid mesostructures displayed no cytotoxicity against eukaryotic cells. It was also demonstrated that the sophorolipid mesostructures loaded with iron oxide nanoparticles could serve as effective hyperthermia agents.



Scheme 43. Synthesis of sophorolipid mesostructures wether or not loaded with iron oxide nanoparticles

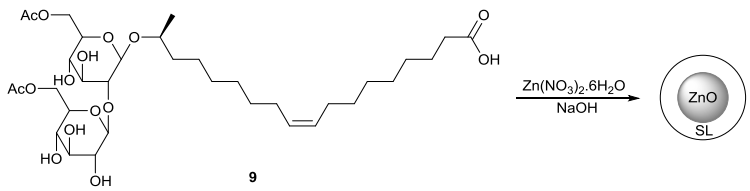
Sophorolipid conjugated cadmium telluride quantum dots (CdTe QD) were synthesized by Singh *et al*. (Scheme 44).[112] In a first step, 3-mercaptopropionic acid was used as the capping agent in the synthesis of the CdTe QDs. Terminal amine groups were introduced *via* an EDC-mediated amidation reaction with ethylenediamine prior to the coupling of diacetylated sophorolipid acid **9**. The obtained nanoparticles were nearly monodispersed with an average particle size of 5 nm for both CdTe QDs **194** and sophorolipid conjugated CdTe QDs **196** *via* TEM analysis. DLS measurements indicated

average particle sizes of 8 and 118 nm for respectively CdTe QDs **194** and sophorolipid conjugated CdTe QDs **196**. Bioimaging studies revealed that sophorolipid conjugated CdTe QDs **196** were taken up in the cytosol of a cancer cell line. Cytotoxicity studies demonstrated a specific toxicity against the cancer cell line compared to a control cell line.



Scheme 44. Synthesis of sophorolipid conjugated cadmium telluride quantum dots

Basak *et al*. described the synthesis of sophorolipid functionalized zinc oxide nanoparticles (Scheme 45).[113] Diacetylated sophorolipid acid **9** was used for the nanoparticle synthesis in combination with zinc nitrate hexahydrate under alkaline conditions. The average particle size was calculated *via* the Debye-Scherrer formula and was found to be 6.55 and 6.02 nm for respectively naked and sophorolipid-capped zinc oxide nanoparticles. The antimicrobial activities of the zinc oxide nanoparticles were evaluated against the Gram-negative bacterium *Salmonella enterica* and the fungus *Candida albicans*. The sophorolipid-capped zinc oxide nanoparticles proved to exhibit a significant inhibitory effect on the growth of both strains at a concentration of 5000 µg/mL. Higher activities were observed for the sophorolipid-capped zinc oxide nanoparticles than for the naked zinc oxide nanoparticles, probably due to the better penetration of the former ones in the pathogenic cells. The mechanism of cell damage on *S. enterica* consisted of cell elongation followed by cell wall disruption. In the case of *C. albicans*, complete cell rupture occurred while cell elongation was not detected. A higher antimicrobial activity was observed against *S. enterica* compared to *C. albicans*.



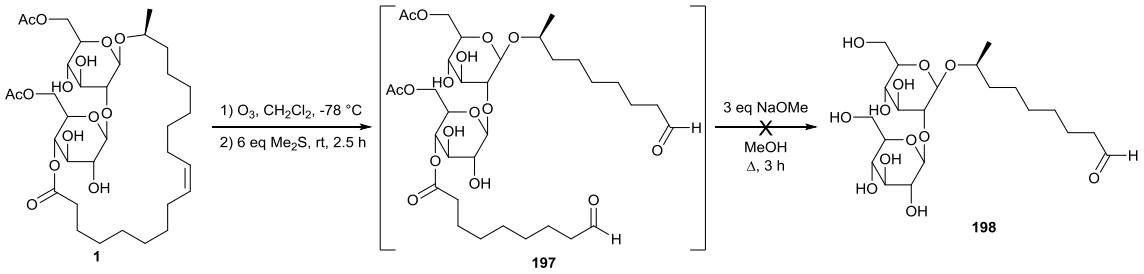
Scheme 45. Sophorolipid-capped zinc oxide nanoparticles

3. Results and discussion

3.1. Synthesis of the intermediate sophorolipid aldehyde

3.1.1. Development of a synthetic pathway towards the sophorolipid aldehyde

In a first attempt to synthesize the desired sophorolipid aldehyde **198**, diacetylated sophorolipid lactone **1** was directly subjected to an ozonolysis reaction (Scheme 46). Safety precautions should be taken during this reaction since the formation of unstable ozonides could result in explosions. Therefore, the reaction was performed in a taped washing flask at -78 °C on a small scale of maximum 30 g. The sophorolipid starting material was dissolved in dry dichloromethane and a pinch of Sudan III dye was added to indicate the end of the reaction. The mixture was purged with ozone until the red color dissipated and flushed with nitrogen to remove all traces of ozone. Dimethyl sulfide was added for the reductive work-up towards the intermediate dialdehyde **197**. The formation of the sophorolipid dialdehyde **197** was demonstrated *via* LC-MS and NMR analysis. Small amounts of the corresponding mono- and dicarboxylic acid derivatives were also detected *via* LC-MS analysis. Purification of the sophorolipid dialdehyde **197** was not successful either *via* column chromatography with ethyl acetate or 5% methanol in dichloromethane as eluent or *via* recrystallization from ethanol. A successful purification was obtained *via* automated column chromatography by applying a gradient of 2 column volumes (CV) at 20:80 ethyl acetate/petroleum ether, 25 CV to 100% ethyl acetate and 5 CV at 100% ethyl acetate. However, a low yield of only 24% was obtained with this purification procedure.



Scheme 46. Synthesis of sophorolipid aldehyde 198 *via* ozonolysis and alkaline methanolysis

Therefore, the crude ozonolysis reaction mixture was directly subjected to an alkaline methanolysis with sodium methoxide in methanol to synthesize the desired sophorolipid aldehyde **198**. After reaction, sophorolipid aldehyde **198** could not be detected *via* LC-MS analysis. Instead, the aldol condensation product **199**, formed from the methyl 9-oxononoate by-product, was detected as the major product, together with small amounts of the C9-sophorolipid acid and the aldol condensation product **200** (Figure 4). The synthesis of the latter compound was confirmed *via* NMR-analysis. These results indicate that the alkaline conditions of the methanolysis reaction are not compatible with the aldehyde functions present in the structure.

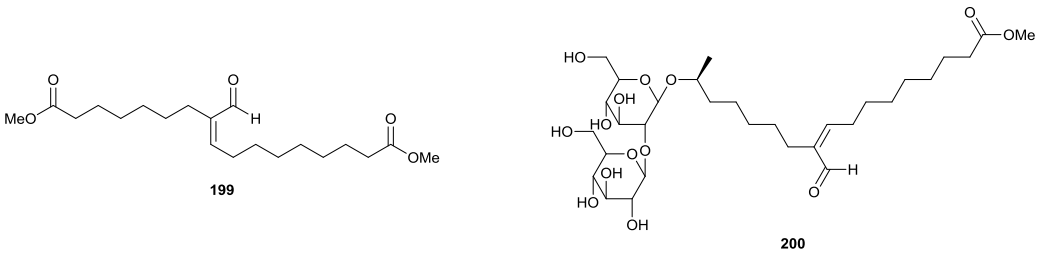
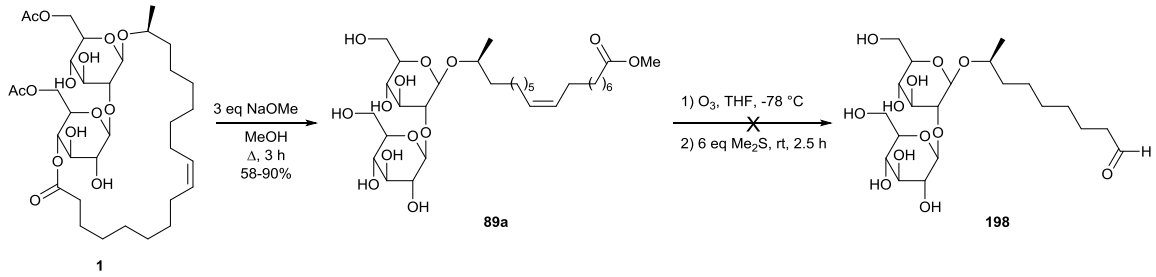


Figure 4. Aldol condensation products after alkaline methanolysis of sophorolipid dialdehyde 197

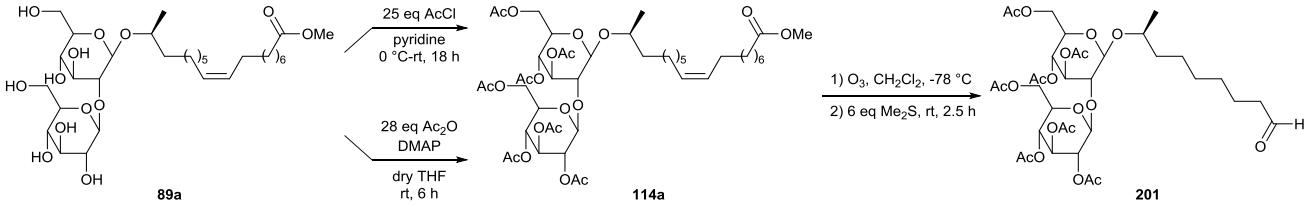
A reversed reaction sequence, *i.e.* alkaline methanolysis of diacetylated sophorolipid lactone **1** followed by an ozonolyis reaction, was evaluated as alternative reaction pathway towards sophorolipid aldehyde **198** (Scheme 47). The alkaline methanolysis was performed with 3 equivalents of sodium methoxide in methanol based on a literature procedure to yield sophorolipid methyl ester **89a**.[74] After reaction, the mixture was acidified with acetic acid and the sophorolipid ester was purified *via* precipitation in water. In subsequent reactions towards sophorolipid methyl ester **89a**, a catalytic amount of sodium methoxide (0.15 equivalents) proved to be sufficient to perform the alkaline methanolysis. In a first attempt to synthesize sophorolipid aldehyde **198** from sophorolipid methyl ester **89a**, the ozonolysis reaction was performed in dichloromethane as previously described. However, no reaction occurred, probably due to the insolubility of sophorolipid methyl ester **89a** in dichloromethane. Tetrahydrofuran was evaluated as alternative solvent since it is also compatible with ozonolysis reactions, but although some reaction occurred, the formation of sophorolipid aldehyde **198** could not be observed.



Scheme 47. Synthesis of sophorolipid aldehyde 198 *via* alkaline methanolysis and ozonolysis

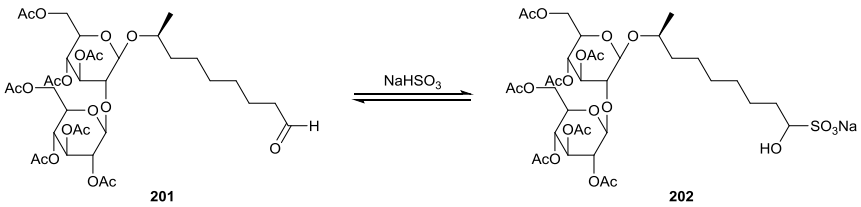
To prevent unwanted side-reactions during the ozonolysis, the sugar head of sophorolipid methyl ester **89a** was protected with acetyl groups. Both acetyl chloride and acetic anhydride were evaluated for this protection step (Scheme 48). Acetylation with 7 equivalents of acetyl chloride in pyridine only resulted in mono-, di-, tri- and tetra-acetylation of sophorolipid methyl ester **89a**. Even with 25 equivalents of acetyl chloride, no complete acetylation could be obtained. Acetylation with acetic anhydride was performed according to a literature procedure and resulted in the complete acetylation towards peracetylated sophorolipid methyl ester **114a**.[89] The amount of acetic anhydride could be reduced to 7.05 equivalents and the reaction time was reduced to 1 hour. Peracetylated sophorolipid methyl ester **114a** was obtained in quantitative yield and high purity after reaction work-up. Further purification *via* column chromatography with a 7:3 mixture of petroleum

ether/ethyl acetate as eluent was performed, resulting in a yield of 76%. However, this purification step had no significant effect on the ozonolysis reaction and was therefore not repeated. Ozonolysis of this protected sophorolipid ester in dichloromethane resulted in the synthesis of the desired sophorolipid aldehyde **201**.



Scheme 48. Acetylation of sophorolipid methyl ester 89a and subsequent ozonolysis towards sophorolipid aldehyde 201

Different methods were evaluated for the purification of sophorolipid aldehyde **201**. At first, the purification was attempted *via* the formation of the sodium bisulfite adduct **202** (Scheme 49). Different solvents, reaction conditions and bases were evaluated for the bisulfite addition and the reverse reaction (Table 1). The best results were obtained for entry 8, but these results were not reproducible. Although it was possible to isolate sophorolipid aldehyde **201** from the reaction mixture in the absence of methyl 9-oxononanoate, the yields were always very low. High amounts of the sodium bisulfite adduct **202** were still present in the organic phase after extraction or filtration, indicating that its solubility in the organic phase is too high. In view of the bad results obtained for the bisulfite addition reaction, this procedure was not suitable for the purification of sophorolipid aldehyde **201**.



Scheme 49. Sophorolipid aldehyde purification *via* the formation of the sodium bisulfite adduct 202

Therefore, it was attempted to purify sophorolipid aldehyde **201** *via* column chromatography. Chromatography on normal phase silica with a 6:4 mixture of petroleum ether/ethyl acetate was performed, resulting in a yield up to 66%. However, large amounts of solvent were needed to elute sophorolipid aldehyde **201** from the column. Other attempts with 5% methanol in dichloromethane as eluent on normal phase silica and a 6:4 mixture of petroleum ether/ethyl acetate on alumina resulted in lower yields (38% and 49% respectively). Therefore, the use of automated column chromatography was evaluated. This alternative purification procedure could drastically reduce the amount of solvent and time necessary for the purification. When a gradient of 1 column volume (CV) at 89:11 petroleum ether/ethyl acetate, 10 CV to 10:90 petroleum ether/ethyl acetate and 1 CV at 10:90 petroleum ether/ethyl acetate was applied, sophorolipid aldehyde **201** could be purified in a

yield up to 67%. The purity of the product could be increased by applying a gradient of 2 CV at 20:80 diethyl ether/hexane, 15 CV to 100% diethyl ether and 8 CV at 100% diethyl ether.

Table 1. Conditions for the bisulfite addition and reverse reaction in the purification of sophorolipid aldehyde 201

	Bisulfite addition					Reverse reaction			
Entry	**$NaHSO_3$**	**Solvent**	**Time**	**Temp.**	**Isolation**	**Base**	**Solvent**	**Time**	**Yield**
1	1.1 eq	EtOAc / H_2O (1:1)	18h	rt	Extraction	4.5 eq K_2CO_3	EtOAc	2h	16%
2	2.2 eq	dry CH_2Cl_2	18h	Δ	Extraction	5 eq K_2CO_3	EtOAc	2h	3%
3	2.2 eq	EtOAc / hexane	18h	30 °C	Filtration	2.5 eq K_2CO_3	EtOAc	2h	8%
4	4.4 eq	MeOH / H_2O (5:1)	90’	rt	Extraction	5 eq K_2CO_3	EtOAc	2h	/
5	4.4 eq	ACN / H_2O (5:1)	90’	rt	Extraction	5 eq K_2CO_3	EtOAc	2h	32%
6	10 eq	EtOAc / H_2O (5:1)	30’ 30’	40 °C 0 °C	Filtration	5 eq K_2CO_3	EtOAc	10’	6%
7	10 eq	ACN / H_2O (5:1)	15’ 45’	40 °C 0 °C	Filtration	5 eq K_2CO_3	H_2O / ACN (5:1)	2h	/
8	5 eq	ACN / H_2O (5:1)	15’ 45’	40 °C 0 °C	Extraction	5 eq $NaHCO_3$	EtOAc	30’	38%
9	10 eq	EtOAc / H_2O (5:1)	15’ 45’	40 °C rt	Extraction	5 eq $NaHCO_3$	EtOAc	30’	46%*
10	10 eq (aq.)**	EtOH	30’	0 °C	Filtration	10 eq $NaHCO_3$	EtOAc	30’	/
11	10 eq (aq.)**	Et_2O	30’	0 °C	Filtration	10 eq $NaHCO_3$	EtOAc	30’	/

* Product was not completely pure after the purification step

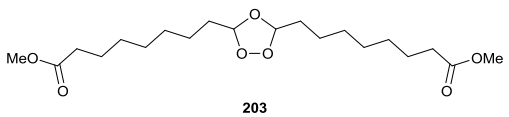
** aq. = aqueous solution

3.1.2. Optimization of the synthetic pathway towards the sophorolipid aldehyde

For the first reaction step, i.e. the alkaline methanolysis with sodium methoxide in methanol, the quality of the sophorolipid starting material proved to be a critical factor. The best results were obtained with sophorolipid lactones derived from fermentations using oleic acid and yeast extract. A *Starmerella bombicola* lactone esterase overexpression strain (oe *sble*) was used for the selective synthesis of diacetylated sophorolipid lactone **1**.[22] Pure sophorolipid methyl ester **89a** could be obtained after precipitation in a yield ranging from 58 to 90%. With sophorolipid lactones from fermentations using canola oil and corn steep liquor (CSL), problems were encountered during the reaction work-up since precipitation of the product did not occur. This lack of precipitation after alkaline methanolysis of sophorolipid lactones from fermentations using canola oil and CSL could be solved by washing the starting product with an aqueous sodium bicarbonate solution after

dissolution in ethyl acetate. Although the lack of precipitation was hereby circumvented, an extra step was added to the synthetic pathway. To determine whether the lack of precipitation was due to the canola oil or CSL, synthesis of sophorolipid methyl ester **89a** was also performed on sophorolipid lactones derived from a fermentation using canola oil and yeast extract. The reaction was performed in parallel with the alkaline methanolysis of sophorolipid lactones from a fermentation using oleic acid and yeast extract on the one hand, and canola oil and CSL on the other hand. For the alkaline methanolysis of the sophorolipid lactones from a fermentation using canola oil and yeast extract, precipitation was obtained after reaction work-up, but the obtained yield was lower compared to product derived from the fermentation using oleic acid (54% versus 70%). Moreover, a more heterogeneous product was obtained, which can be expected since canola oil still contains other fatty acids next to oleic acid. Diacetylated sophorolipid lactones obtained from fermentations using oleic acid and yeast extract are thus clearly more suitable for this synthetic pathway. On the one hand, the use of yeast extract is necessary to obtain precipitation of sophorolipid methyl ester **89a** after reaction work-up. On the other hand, oleic acid is preferred to obtain a higher yield and a more homogeneous product.

At first, the ozonolysis reaction was performed in dichloromethane and dimethyl sulfide was used for the reductive work-up towards sophorolipid aldehyde **201**. Although the desired product could be isolated in high yield, the methyl 9-oxononanoate by-product could not be detected *via* LC-MS analysis. Instead, only the presence of the stable 1,2,4-trioxolane (ozonide) intermediate **203** was observed (Figure 5). The presence of high concentrations of residual ozonides after reaction work-up with dimethyl sulfide has already been described by the research group of Patrick Dussault.[114] As the isolation of methyl 9-oxononanoate is most desirable for a green synthetic pathway, the formation of the ozonide intermediate **203** had to be prevented. This would have a positive effect on green chemistry metrics such as the environmental (E) factor and the atom economy.

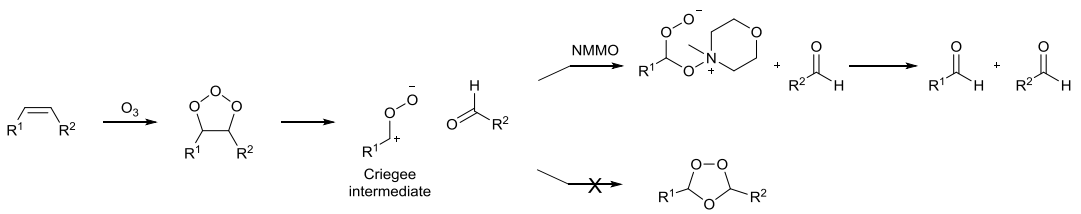


203

Figure 5. Methyl 9-oxononanoate derived ozonide intermediate 203

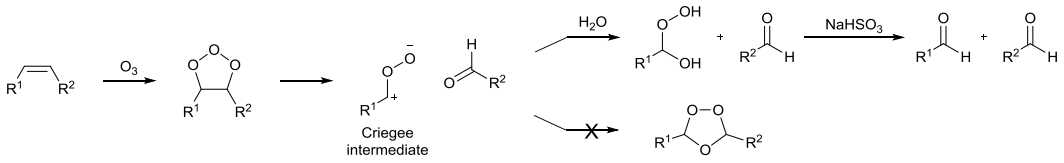
Different procedures were evaluated to prevent the formation of the ozonide intermediate **203**. All procedures are based on trapping the Criegee intermediate which is formed after decomposition of the primary ozonide. In a first attempt, 3 equivalents of *N*-methylmorpholine *N*-oxide were added to the ozonolysis reaction mixture (Scheme 50).[114] The amine oxide can react with the Criegee intermediate to form a tetrahedral intermediate. Fragmentation of this intermediate will yield the desired aldehyde product. Work-up was performed by adding 1 equivalent of acetic acid and washing

the reaction mixture with water. However, both with dichloromethane and acetonitrile as solvent at 0 °C, no complete reaction towards sophorolipid aldehyde **201** was obtained.



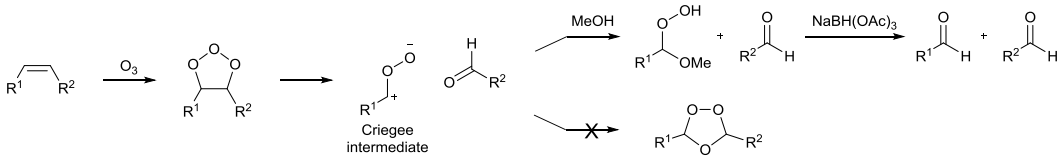
Scheme 50. Ozonolysis reaction in the presence of *N*-methylmorpholine *N*-oxide

Also water was evaluated as trapping agent. Therefore, the ozonolysis reaction was performed in a mixture of 5% water in acetonitrile at 0 °C (Scheme 51).[115] After the reaction, 1 equivalent of sodium bisulfite was added to destroy the peroxide formed during the reaction. Although complete conversion was obtained with this procedure, the formation of the ozonide intermediate **203** still occurred.



Scheme 51. Ozonolysis reaction in the presence of water

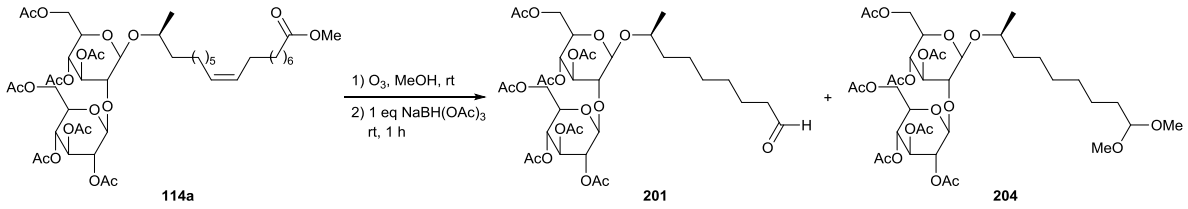
A final attempt comprised the use of methanol as trapping agent. Methanol (1 equivalent) was added to the reaction mixture in order to trap the Criegee intermediate as a hydroperoxyacetal (Scheme 52).[116] Reduction of the hydroperoxyacetal with sodium triacetoxyborohydride furnished the desired aldehyde. This procedure proved to be successful to prevent the formation of the ozonide intermediate **203**. Care should be taken to add exactly 1 equivalent of sodium triacetoxyborohydride for the reductive work-up. In case more reducing agent was added, reduction towards the sophorolipid alcohol derivative could be detected as well *via* LC-MS analysis.



Scheme 52. Ozonolysis reaction in the presence of methanol

To develop a greener synthetic pathway, it was attempted to use methanol as solvent for the ozonolysis reaction in order to replace dichloromethane which is classified as a hazardous solvent. Good results were obtained for the first trials performed with product from small scale fermentation batches (3 L) using oleic acid and canola oil. The reaction temperature was first raised to 0 °C and later on to room temperature to avoid solubility problems which occurred at lower temperatures. To ensure safe reaction conditions, the flask was taped during the reaction. However, when the

ozonolysis reactions were performed with product derived from a fermentation using canola oil and CSL, the formation of an unwanted sophorolipid methyl acetal **204** was observed as well (Scheme 53). The amount of sophorolipid methyl acetal **204** in the reaction product was estimated to be 25-40% based on NMR analysis. Automated flash chromatography of the ozonolysis product did not result in the separation of sophorolipid aldehyde **201** from sophorolipid methyl acetal **204**. The transformation of sophorolipid methyl acetal **204** back into sophorolipid aldehyde **201** was attempted with trifluoroacetic acid in a mixture of ethyl acetate and water, but was not always reproducible. The addition of oxalic acid pretreated silica was also not successful.[117] Only stirring the reaction mixture for 4 hours with a 2 N hydrochloric acid solution in ethyl acetate could convert sophorolipid acetal **204** back to the desired sophorolipid aldehyde **201**. However, a lower yield (only 48%) was obtained after purification.

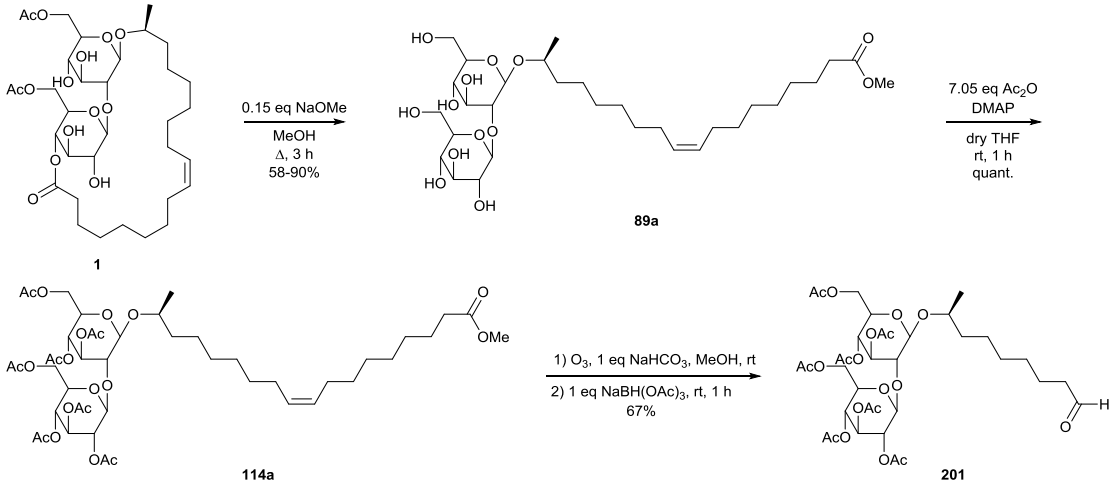


Scheme 53. Synthesis of sophorolipid methyl acetal 205 during the ozonolysis reaction

As the problems arose with product from the fermentation using canola oil and CSL, it was anticipated that the fermentation substrate caused the formation of sophorolipid acetal **204**. However, when the ozonolysis was performed again on peracetylated sophorolipid methyl ester **114a** derived from an oleic acid fermentation, the formation of sophorolipid acetal **204** also occurred. This demonstrated that the acetal formation is not related to variations in the fermentation substrate. Since a catalytic amount of acid is generally used to induce acetal formation, it was assumed that the only remaining explanation was that some traces of acid were responsible for the formation of the unwanted sophorolipid acetal **204**. To prove this, addition of sodium bicarbonate to the ozonolysis reaction was attempted in order to neutralize any traces of acid. With this new reaction procedure, the presence of sophorolipid acetal **204** in the reaction mixture was indeed no longer observed. The origin of the trace of acid is still unknown. The presence of acetic acid seems the most plausible option, deriving from the previous acetylation reaction with acetic anhydride or exempted from the sodium triacetoxyborohydride reducing agent. However, in one particular case where 1 equivalent of acetic acid was deliberately added, acetal formation was not observed. Nevertheless, only traces of acid can catalyze the formation of the sophorolipid acetal **204**.

Ethanol and isopropanol were also evaluated as solvents for the ozonolysis reaction. The use of these solvents could not prevent the acetal formation in the absence of sodium bicarbonate. Moreover, the Sudan III dye was not easily soluble in both solvents, which raised problems to determine the end

of the reaction. Therefore, methanol is the most suitable solvent for the ozonolysis reaction. However, a slight increase of impurities present in the ozonolysis reaction product could be detected when methanol was used as solvent instead of dichloromethane, overoxidation to the corresponding sophorolipid acid occurred more easily and slightly lower yields were generally obtained *via* automated column chromatography. The less visible color change in methanol than in dichloromethane could be the cause for the overoxidation. The optimized synthetic pathway for the synthesis of sophorolipid aldehyde **201** is depicted in Scheme 54.



Scheme 54. Optimized synthetic pathway towards sophorolipid aldehyde 201

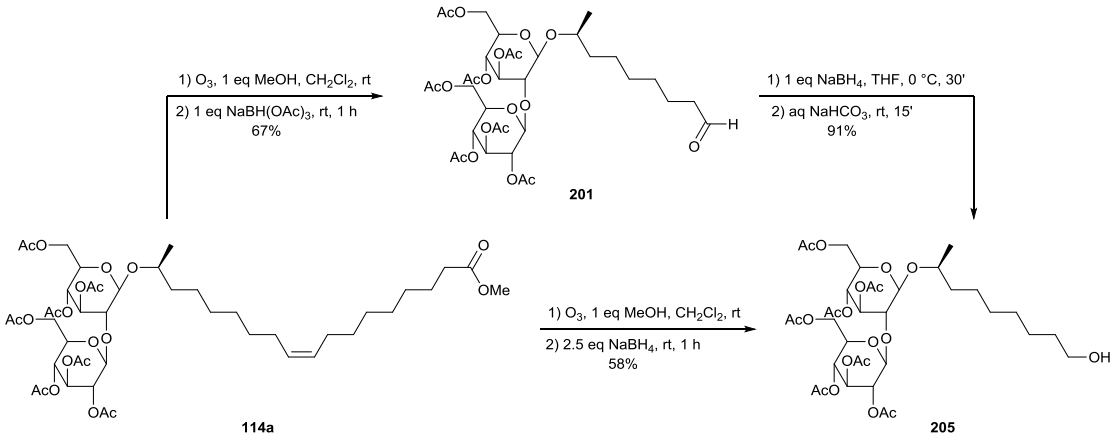
Different green chemistry metrics were evaluated for the optimized synthetic pathway towards sophorolipid aldehyde **201** (Table 2).[118] Two scenarios are evaluated, namely the isolation of only sophorolipid aldehyde **201** as desired product (Scenario A) which is compared to the isolation of sophorolipid aldehyde **201** together with methyl 9-oxononanoate as a valuable by-product (Scenario B). A first metric is the carbon efficiency (CE) which estimates the percentage of carbon from the reagents that is contained in the final product. This metric does not take into account the amount of solvent used and waste generated during the process such as the amount of solvent. A second metric is the atom economy (AE) which estimates the total amount of reagents that are incorporated into the final product. Also here, only the reagents which are incorporated into the final product are taken into account. Solvent use and waste generation are not quantified. A third metric is the reaction mass efficiency (RME) which takes into account the reaction yield, the atom economy, the stoichiometry of the reaction and the amount of waste products. This metric takes into account all process conditions and therefore offers a good estimation of the greenness of the overall synthetic pathway. The RME is inversely related to environmental (E-) factor. This fourth metric estimates the amount of waste produced for the synthesis of a certain amount of product. The E-factor thus also offers a good estimation of the greenness of the overall synthetic pathway. The calculated E-factors lie in the range of those for fine chemicals (5-50 kg/kg).[118]

Table 2. Overview of the green chemistry metrics for the production of only sophorolipid aldehyde 201 (Scenario A) and its production in combination with the isolation of the methyl 9-oxononanoate by-product (Scenario B). CE = carbon efficiency, AE = atom economy, SF = stoichiometric factor, RME = reaction mass efficiency, ε = reaction yield, c = mass reaction catalyst, s = mass reaction solvent, w = mass reaction waste, m = mass target product.

Parameter	Formula	Scenario A	Scenario B
CE (%)	$\frac{(\text{Amount of carbon in product}) * 100}{\text{Total amount of carbon in reagents}}$	54	69
AE (%)	$\frac{(\text{Molecular weigth of product}) * 100}{\sum \text{Molecular weigth of reagents}}$	50	62
SF	$1 + \frac{(\text{AE}) * \sum \text{mass of excess reagent}}{\text{Expected product mass at 100\% yield}}$	1.0033	1.0032
RME (%)	$\varepsilon * (\text{AE}) * \frac{1}{\text{SF}} * \left[\frac{1}{1 + \frac{\varepsilon * (\text{AE}) * (\text{c} + \text{s} + \text{w})}{(\text{SF}) * \text{m}}}\right]$	0.048	0.060
E-factor (kg/kg)	$\frac{\text{Mass waste}}{\text{Mass product}}$	17.55	9.53

3.1.3. Synthesis of a sophorolipid alcohol intermediate

In the course of the optimization of the synthetic pathway towards the sophorolipid aldehyde, the synthesis of sophorolipid alcohol **204** was also attempted (Scheme 55). When the reduction of sophorolipid aldehyde with sodium borohydride was performed in methanol, only the deprotected sophorolipid alcohol could be detected by LC-MS analysis. Changing the solvent to tetrahydrofuran solved this problem and sophorolipid alcohol **205** could be isolated in a high yield of 91%. The direct synthesis of sophorolipid alcohol **205** from peracetylated sophorolipid methyl ester **114a** could also be performed by using sodium borohydride instead of sodium triacetoxyborohydride for the reductive work-up after the ozonolysis reaction. When 2 equivalents of the reducing agent were used, the presence of sophorolipid aldehyde **201** was also detected. Therefore, 2.5 equivalents had to be used to obtain complete reduction. Sophorolipid alcohol **205** was purified *via* automated column chromatography by applying a step-wise gradient of 10 column volumes (CV) at 20:80 ethyl acetate/petroleum ether, 10 CV at 30:70 ethyl acetate/petroleum ether, 20 CV at 40:60 ethyl acetate/petroleum ether, 5 CV at 80:20 ethyl acetate/petroleum ether and 5 CV at 90:10 ethyl acetate/petroleum ether. The yield of the purified sophorolipid alcohol **205** was 58%. However, this purification was not further optimized.



Scheme 55. Synthesis of intermediate sophorolipid alcohol 204

3.1.4. Incorporation of petroselinic acid in sophorolipid derivatives towards a C12 sophorolipid aldehyde

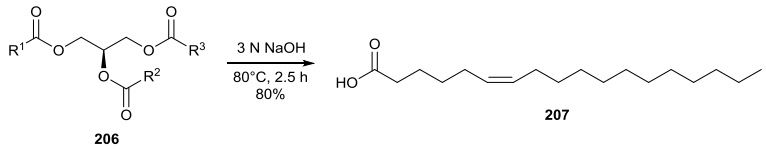
In the previous section, the development of a synthetic pathway towards the C9 sophorolipid aldehyde **201** is described. This procedure was extended to the production of a C12 sophorolipid aldehyde *via* the incorporation of petroselinic acid in the sophorolipid structure.

Petroselinic acid is a rather uncommon fatty acid. With its double bond at the 6,7-position, it is a positional isomer of oleic acid. The position of this double bond influences the properties of the fatty acid. For example, the melting point of petroselinic acid is 30 °C, while the melting point of oleic acid is only 14 °C.[119] Petroselinic acid is found in high amounts in the seed oils from plants belonging to the Apiaceae family, also known as Umbelliferae, and the Araliaceae family.[120] The quantity of petroselinic acid varies from 31 to 75% in the vegetable oil of fruits from *Coriandrum sativum*, one of the most enriched sources of petroselinic acid. This vegetable oil can be extracted from the fruits *via* twin-screw extrusion.[121] Petroselinic acid is already applied in cosmetic formulations as a moisturizing and anti-aging agent, and as a skin-irritation reducing agent in α-hydroxy acid containing compositions.[122-124] Besides, a considerable antimicrobial activity against several bacteria, yeast and mold species was observed.[120] Several modifications of petroselinic acid are described, among others towards surfactants and the nylon 66 precursor adipic acid.[119-120, 125] When incorporated in triglycerides, lipolysis by pancreatic lipase occurs at a much lower efficiency than for oleic acid triglycerides.[119, 126] Therefore, petroselinic acid rich oils may offer a low-fat alternative for conventional vegetable oils. It was also suggested that petroselinic acid inhibits the synthesis of arachidonic acid, which could counteract the vasoconstrictive effects related to arachidonic acid overproduction.[127-128]

Being a structural isomer of oleic acid, petroselinic acid could be applied for the synthesis of a new type of sophorolipids. As the properties for petroselinic acid differ considerably from those of oleic

acid, it can be anticipated that petroselinic acid derived sophorolipids will possess different biological activities and self-assembly properties than the oleic acid based sophorolipids. Moreover, when the previously described synthetic pathway is applied to the petroselinic acid based sophorolipids, the synthesis of a C12 sophorolipid aldehyde can be accomplished.

As mentioned before, petroselinic acid is the major fatty acid in the vegetable oil of *Coriandrum sativum* fruits. This vegetable oil was isolated *via* twin-screw extrusion at the Laboratoire de Chimie Agro-industrielle (ENSIACET, Université de Toulouse) *via* a previously reported procedure.[121] The identification of the different fatty acids and their distribution in the vegetable oil was determined *via* gas chromatography analysis of the fatty acid methyl esters at the same research group (Table 3). Petroselinic acid is clearly the major fatty acid, constituting 73.3% of all fatty acids. Linoleic acid and oleic acid are present in a lower amount, respectively 13.9% and 5% of all fatty acids. The triglycerides from the vegetable oil were hydrolyzed into glycerol and a fatty acid mixture *via* an alkaline hydrolysis with sodium hydroxide (Scheme 56). Petroselinic acid **207** was isolated from the reaction mixture *via* crystallization in absolute ethanol. A high yield of 80% was obtained, based on the amount of petroselinic acid present in the vegetable oil.



206 207

Scheme 56. Alkaline hydrolysis towards petroselinic acid

Table 3. Fatty acid composition (%) of the vegetable oil of Coriandrium sativum fruits. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n.d. = not detected.

Fatty acid	Content (%)
Caproic acid (C6:0)	*n.d.*
Myristic acid (C14:0)	0.2
Palmitic acid (C16:0)	3.3
Palmitoleic acid (C16:1)	0.2
Margaric acid (C17:0)	*n.d.*
Stearic acid (C18:0)	0.7
Petroselinic acid (C18:1n-12)	**73.3**
Oleic acid (C18:1n-9)	5.0
cis-Vaccenic acid (C18:1n-7)	1.3
Linoleic acid (C18:2)	13.9
Linolenic acid (C18:3)	0.1
Arachidic acid (C20:0)	0.1
Gadoleic acid (C20:1)	*n.d.*
SFA	4.2
MUFA	79.9
PUFA	14.0
Identified fatty acids	98.1

Petroselinic acid **207** was then used as substrate for microbial sophorolipid production. The sophorolipid fermentation was performed in cooperation with the InBio research group (Ghent University). A *Starmerella bombicola* lactone esterase overexpression strain (oe *sble*) was used for the selective synthesis of diacetylated sophorolipid lactone **208**.[22] The *S. bombicola* oe *sble* strain was cultivated on Lang medium (132 g/L glucose monohydrate; 4g/L yeast extract; 5 g/L sodium citrate tribasic dihydrate; 1.5 g/L NH_4Cl; 1 g/L KH_2PO_4; 0.16 g/L K_2HPO_4; 0.7 g/L $MgSO_4 \cdot 7H_2O$; 0.5 g/L NaCl; 0.27 g/L $CaCl_2 \cdot 2H_2O$) in a Biostat® B 3 L culture vessel (Sartorius-BBI Systems) with a working volume of 1.1 L. Temperature (30 °C), pH (3.5), stirring rate, and air flow rate (1.5 L/min) were controlled by the Biostat® B control unit.[129] 100 mL of 30 h old shake flask cultures was used for inoculation of the culture vessel. 20 g of petroselinic acid was added to the reactor just after inoculation. From then on, an extra portion of 5 g petroselinic acid was added every 24 h. The fermentation parameters are depicted in Figure 6. The initial pH of 5.8 was allowed to drop spontaneously till 3.5 and was maintained at this value afterwards by automated addition of a 5 N NaOH solution. After 147 h of inoculation, glucose was almost completely depleted upon which additional glucose was added to the medium to ensure optimal sophorolipid production. After 18 h, the stirring rate was raised to 700 rpm since the dissolved oxygen (pO_2) dropped to 3%. After 163 h, the stirring rate was raised once more to 800 rpm since the produced sophorolipid lactones formed a highly viscous broth. Maintaining the dissolved oxygen at an adequate level is crucial for the sophorolipid production, since the cytochrome P450 monooxygenase enzyme requires aerobic conditions. The growth of the culture was monitored by measuring the optical density (OD). However, the determination of the amount of colony forming units (CFU) would have been more informative since residual fatty acids and produced sophorolipids can interfere with the OD measurement.

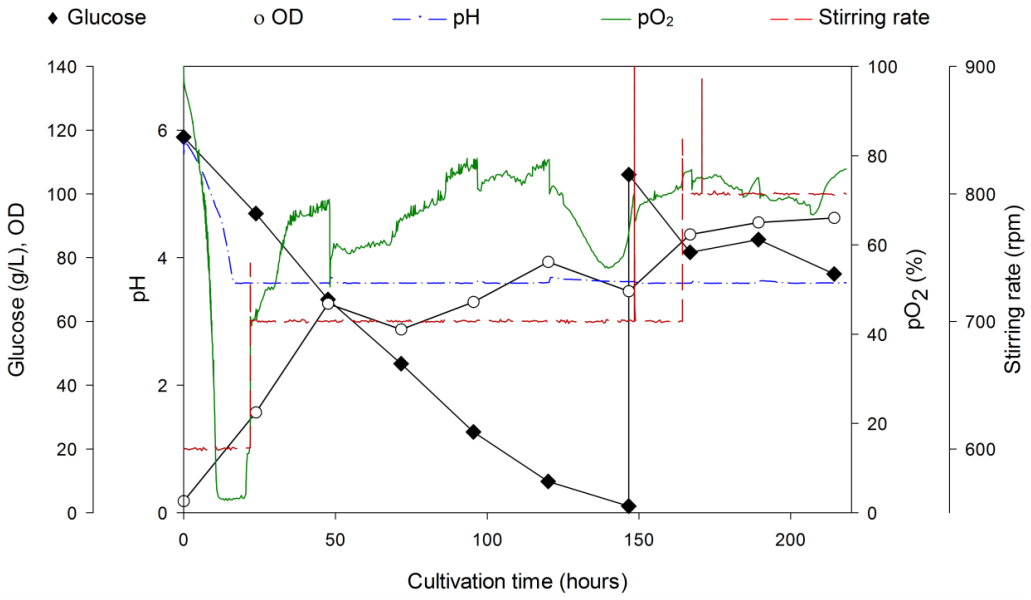


Figure 6. Sophorolipid fermentation parameters depicted in function of time

The fermentation broth was transferred to a 2 L flask and the culture vessel was rinsed with 500 mL water which was also collected (Figure 7). The fermentation broth was kept overnight at 50 °C to induce precipitation of the sophorolipid lactones. Afterwards, the upper water layer which contains the yeast cells was removed, filtered over a Whatman filter and once more kept overnight at 50 °C to induce precipitation of residual sophorolipid lactones. The sophorolipid fraction was suspended in water, transferred to a 2 L erlenmeyer and shaken overnight at 4 °C to induce crystallization of the sophorolipid lactones. However, crystallization did not occur as is the case for oleic acid based sophorolipid lactones. Therefore, the dense sophorolipid phase was separated from the water phase, dissolved in ethyl acetate and washed with an aqueous sodium bicarbonate solution. The ethyl acetate phase was dried over magnesium sulfate and concentrated under reduced pressure. The water phase was also extracted with ethyl acetate, washed with sodium bicarbonate, dried over magnesium sulfate and concentrated under reduced pressure. After the downstream processing, a total amount of 44 g was obtained as a white powder from the combined ethyl acetate fractions. This corresponds to a total production of 40 g/L.

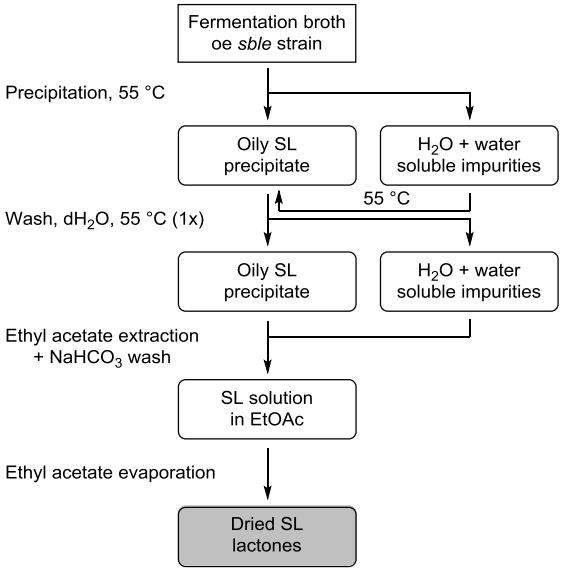


Figure 7. Downstream processing for sophorolipid purification

Pure diacetylated sophorolipid lactone **208** was obtained after the downstream processing as a white powder. Incorporation of *de novo* synthesized fatty acids such as oleic acid was not observed *via* NMR-analysis. Comparison of oleic acid based sophorolipid lactone **1** and petroselinic acid based sophorolipid lactone **208** *via* ^{13}C-NMR clearly demonstrates the difference between the two sophorolipid compounds (Figure 8). This difference can be seen most clearly by comparing the peaks for the double bond carbon atoms. The difference in ppm value for both carbon peaks is much bigger for petroselinic based sophorolipid lactone **208** than for oleic acid based sophorolipid **1**. Moreover, the presence of a minor fraction of another double bond moiety can be detected for oleic acid based

sophorolipid **1** which is not the case for petroselinic based sophorolipid lactone **208**, indicating a higher purity for the latter compound.

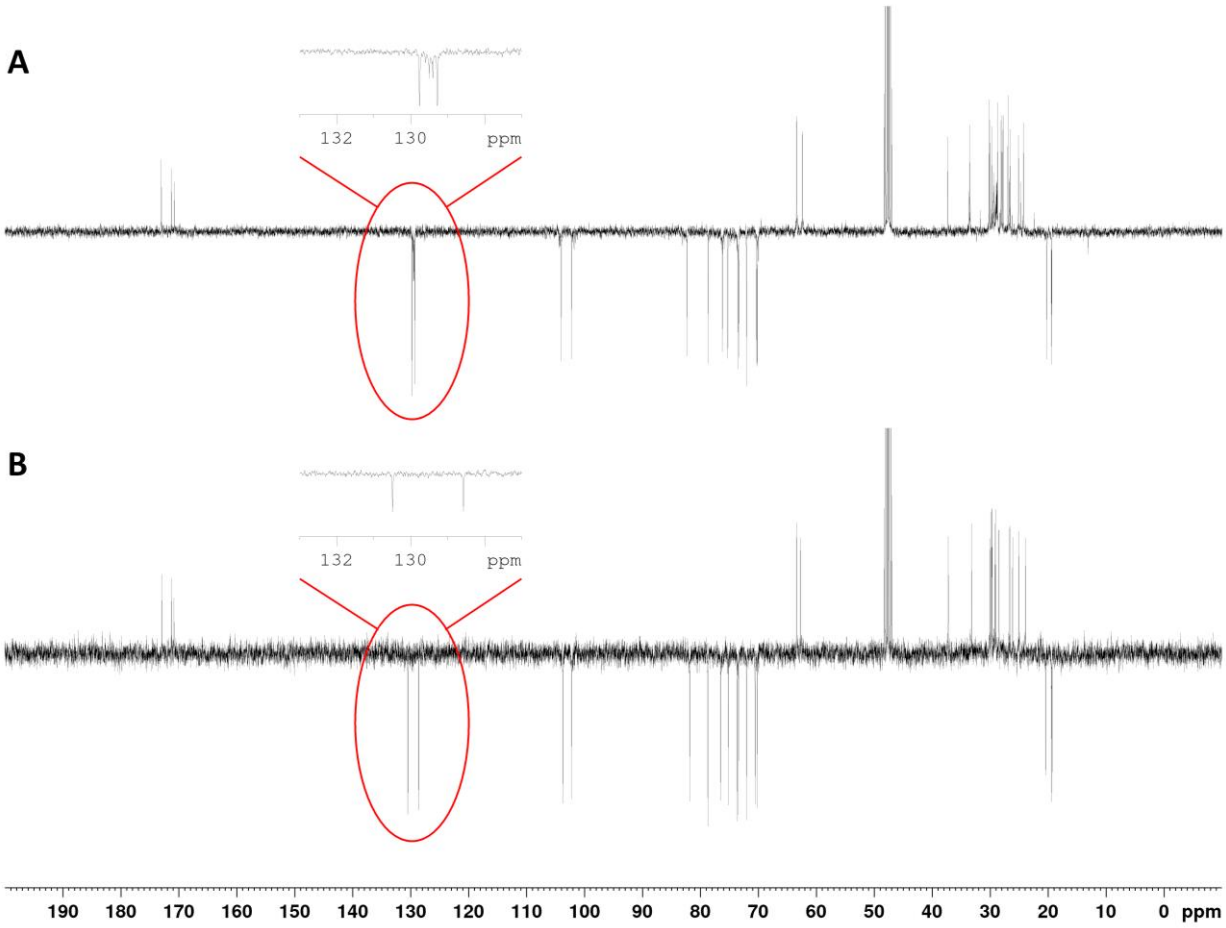
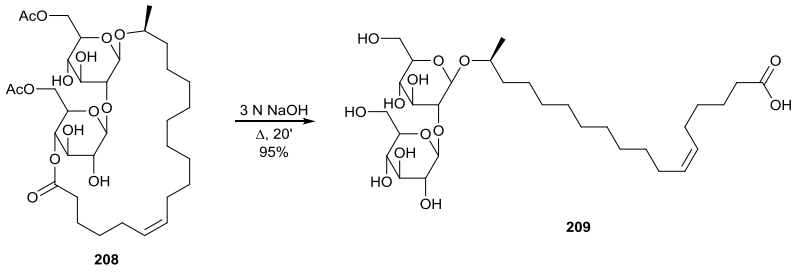


Figure 8. Comparison of oleic acid based sophorolipid lactone (A) and petroselinic acid based sophorolipid lactone (B) by ^{13}C NMR

Diacetylated sophorolipid lactone **208** was subsequently subjected to alkaline hydrolysis with NaOH to yield petroselinic acid based sophorolipid acid **209** (Scheme 57). For both petroselinic acid based sophorolipid compounds, the critical micelle concentration (CMC) value and the corresponding surface tension were determined and compared to their oleic acid based counterparts (Table 4). The CMC of a surfactant is the lowest concentration for which micelles are formed. The surface tension measurements were determined in cooperation with the Particle and Interfacial Technology Group (Ghent University). Much lower CMC values are obtained for the petroselinic acid based sophorolipids compared to their oleic acid based counterparts. However, the minimal surface tension at these CMC values is almost the same. A different spatial orientation of the hydrophilic and hydrophobic parts of the petroselinic acid based sophorolipids, due to which they experience less steric hindrance and repulsion, could explain their lower CMC values compared to their oleic acid based counterparts. After all, when a certain compound experiences more repulsion and the molecules can approach less close to each other, the air-water interface will be completely saturated

at a lower concentration, resulting in the formation of micelles at a lower concentration. To evaluate this hypothesis, the 3D structures of the oleic and petroselinic acid based sophorolipid lactones were simulated with the software Chem3D Pro 14.0 (Figure 9). These simulations support the hypothesis, since the hydrophilic and hydrophobic regions of the petroselinic acid based sophorolipid lactone appear to occupy a bigger area than those of the oleic acid based sophorolipid lactone. One should take into account that these simulations are only a first indication of the 3D structure, since quantum chemical calculations are necessary to obtain a realistic simulation of the standard geometry for each structure. However, a further investigation of the geometry of these compounds falls without the scope of this project. The increasing hydrophobicity for petroselinic acid based sophorolipid lactone compared to petroselinic acid based sophorolipid acid resulted in a decreasing CMC value, as was the case for their oleic acid based counterparts.



208 209

Scheme 57. Alkaline hydrolysis towards petroselinic acid based sophorolipid acid 209

Table 4. Comparison of the critical micelle concentration and corresponding surface tension for petroselinic acid (PA) and oleic acid (OA) based sophorolipid compounds

	CMC (mg/L)	Surface tension (mN/m)
PA SL lactone	4.2 ± 0.1	34.3 ± 0.0
OA SL lactone	45.1 ± 0.1	33.9 ± 0.7
PA SL acid	154 ± 5	42.0 ± 0.3
OA SL acid	245 ± 9	40.9 ± 0.3

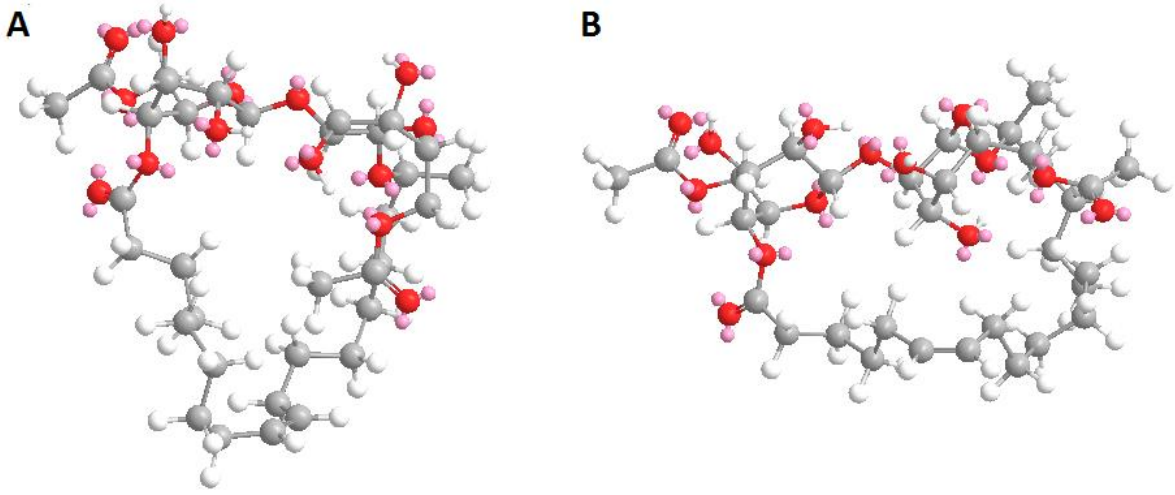
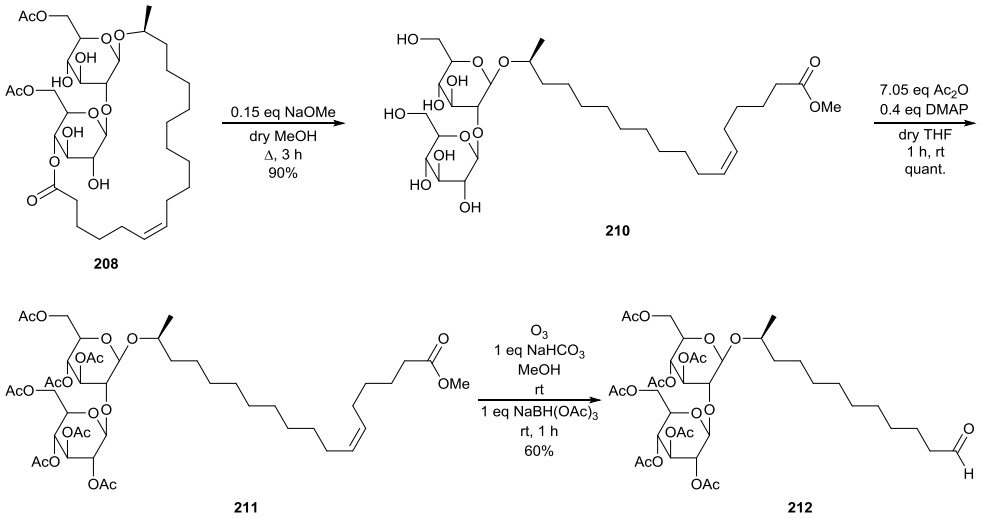


Figure 9. Simulation of the 3D structure of oleic acid based sophorolipid lactone (A) and petroselinic acid based sophorolipid lactone (B) with the software Chem3D Pro 14.0 after minimization of the energy for each structure

The previously described synthetic pathway was applied to the petroselinic acid based diacetylated sophorolipid lactone **208** for the synthesis of the C12 sophorolipid aldehyde **212** (Scheme 58). Sophorolipid lactone **208** was transformed into sophorolipid methyl ester **210** *via* alkaline methanolysis with 0.15 equivalents of sodium methoxide in methanol. Protection of the sugar head group *via* acetylation with acetic anhydride yielded peracetylated sophorolipid methyl ester **211**. Cleavage of the double bond was obtained *via* ozonolysis in methanol with 1 equivalent of sodium bicarbonate and reductive work-up with 1 equivalent of sodium triacetoxyborohydride. C12 sophorolipid aldehyde **212** was purified *via* automated column chromatography using a gradient of 2 CV at 20:80 diethyl ether/hexane, 15 CV to 100% diethyl ether and 9 CV at 100% diethyl ether.



Scheme 58. Chemical modification towards sophorolipid aldehyde 212

3.1.5. Conclusions

A synthetic pathway towards the desired sophorolipid aldehyde intermediate was successfully developed. The influence of the fermentation conditions and the concomitant purity of the starting product on the chemical derivatization proved to be a first critical factor. Diacetylated sophorolipid lactones obtained from fermentations with a *Starmerella bombicola* oe *sble* strain using oleic acid and yeast extract as substrate were most suitable as starting product. On the one hand, the use of this modified yeast strain and oleic acid as substrate is preferred to obtain a higher yield and a more homogeneous product. On the other hand, the use of yeast extract is necessary to obtain precipitation of the sophorolipid methyl ester **89a** after reaction work-up.

The ozonolysis reaction proved to be a second critical factor and can be addressed as the bottleneck of the synthetic pathway. In the optimized reaction procedure, the isolation of methyl 9-oxononanoate as a valuable by-product was enabled and methanol is used as solvent instead of dichloromethane to increase the green character of the synthetic pathway. However, a slight

increase of impurities present in the ozonolysis reaction product could be detected, *i.e.* overoxidation to the corresponding sophorolipid acid occurred more easily and slightly lower yields were generally obtained *via* automated column chromatography. Green chemistry metrics were calculated for two scenarios where wheter or not the islation of the methyl 9-oxononanoate by-product was taken into account. For both scenarios, an E-factor was obtained which lies in the range of E-factors for fine chemicals. Future work could focus on the use of continuous flow microreactor technology to ensure a higher control of the reaction parameters, resulting in a higher selectivity and an increase in process efficiency and safety. Moreover, it would enable the safe-scale up, eventually to an industrial scale, of this potential dangerous reaction.

The synthetic pathway was extended to the production of a sophorolipid alcohol intermediate by adjusting the reductive work-up of the ozonolysis reaction and to the production of a C12 sophorolipid aldehyde *via* the incorporation of petroselinic acid in the sophorolipid structure. Therefore, this synthetic pathway resulted in the successful synthesis of three sophorolipid intermediates, i.e. sophorolipid aldehyde **201**, sophorolipid alcohol **204**, and C12 sophorolipid aldehyde **212** (Figure 10).

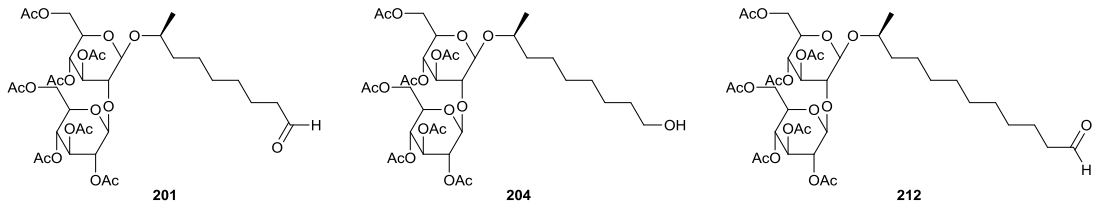


Figure 10. Intermediate sophorolipid derivatives

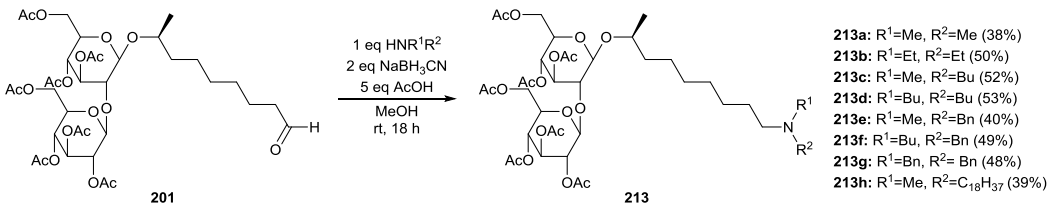
3.2. Modification of the intermediate sophorolipid aldehyde towards new sophorolipid derivatives

3.2.1. Reductive amination towards sophorolipid amines

The intermediate sophorolipid aldehyde **201** was used for the formation of a broad set of innovative sophorolipid derivatives. The first step in this modification pathway comprised the synthesis of sophorolipid amines *via* reductive amination.

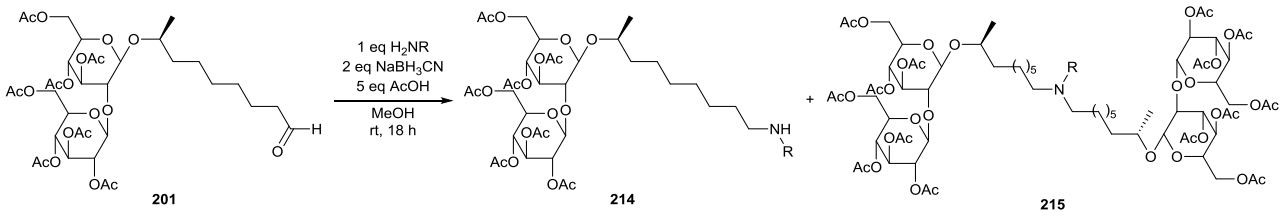
The reductive amination with a variety of secondary amines was evaluated (Scheme 59). Sophorolipid aldehyde **201** was dissolved in methanol, 1 equivalent of secondary amine, 2 equivalents of sodium cyanoborohydride and 5 equivalents of acetic acid were added and the mixture was stirred overnight at room temperature. After evaporation of the solvent, the reaction product was dissolved in ethyl acetate for a washing step with sodium bicarbonate. When the reductive amination was performed with highly pure sophorolipid aldehyde **201**, sophorolipid amines **213** were obtained in high purity and no extra purification step was needed. However, further purification proved to be necessary to obtain highly pure product for further modification reactions when less pure sophorolipid aldehyde **201** was used.

At first, the purification was attempted *via* acid-base extraction. After evaporation of the solvent, the reaction product was dissolved in ethyl acetate and 5 equivalents of acetic acid were additionally added. The mixture was stirred for 30 minutes and subsequently extracted with water. The combined water fractions were stirred for 30 minutes with 10 equivalents of sodium bicarbonate and subsequently extracted with ethyl acetate to isolate sophorolipid amines **213**. However, the isolated yields were very low (1-50%), probably due to the hydrophobic character of sophorolipid amines **213** which is induced by the acetyl groups. Moreover, this purification step generally did not result in an increased purity of the product. Therefore, automated column chromatography was also evaluated for the purification of sophorolipid amines **213**. When a gradient with ethyl acetate/petroleum ether as eluent was applied, tailing of the sophorolipid amines on the column was observed. This tailing could be avoided by adding triethylamine to the eluent, resulting in the complete purification of the different sophorolipid amine derivatives.

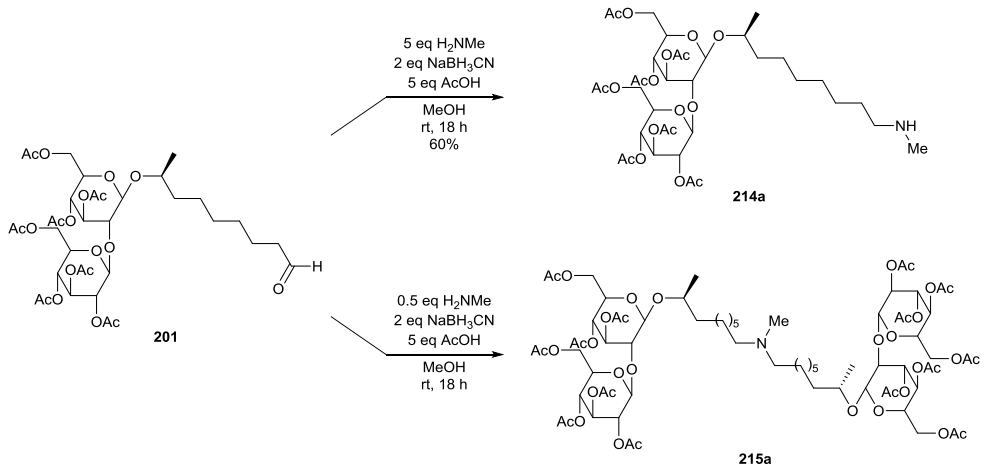


Scheme 59. Reductive amination of sophorolipid aldehyde 201 towards sophorolipid amines 213

Reductive amination with primary amines was also evaluated. When the same reaction conditions were applied as for the reductive amination with secondary amines, a mixture of secondary sophorolipid amines **214** and bolaamphiphilic sophorolipid amines **215** was obtained (Scheme 60). The selective formation of either secondary sophorolipid amines **214** or bolaamphiphilic sophorolipid amines **216** was evaluated with methylamine (Scheme 61). For the reductive amination towards *N*-methyl sophorolipid amine **214a**, 5 equivalents of methylamine were used and the mixture of sophorolipid aldehyde **201** and methylamine was stirred for 1 hour at room temperature prior to the addition of sodium cyanoborohydride and acetic acid. *N*-methyl sophorolipid amine **214a** was obtained in high purity after reaction work-up and did not require further purification. *N*-methyl bolaamphiphilic sophorolipid amine **215a** was selectively formed by using only 0.5 equivalents of methylamine and applying the same reaction conditions as described for the reductive amination with the secondary amines. The synthesis of more bolaamphiphilic sophorolipid amines **215** and their purification will be described in chapter 3.2.4. (Synthesis of bolaamphiphilic sophorolipids).

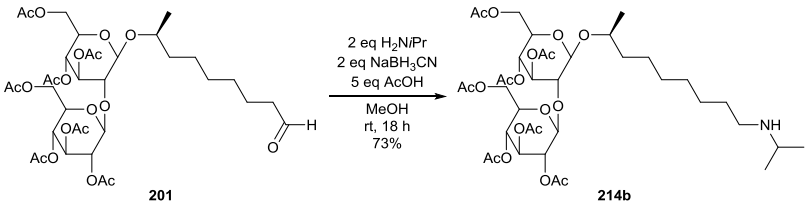


Scheme 60. Reductive amination with primary amines towards a mixture of secondary sophorolipid amines 214 and bolaamphiphilic sophorolipid amines 215



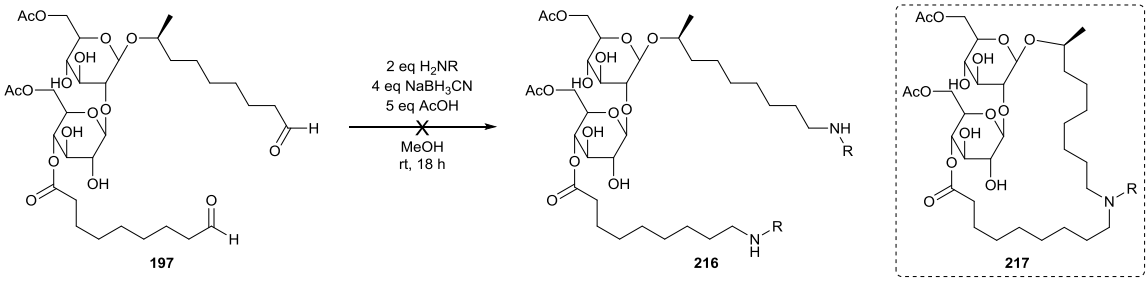
Scheme 61. Selective formation of *N*-methyl sophorolipid amine 214a and *N*-methyl bolaamphiphilic sophorolipid 215a

The reductive amination with isopropylamine was also evaluated (Scheme 62). In contrast to the reductive amination with methylamine, sodium borohydride and acetic acid could be added together with 2 equivalents of the amine. An increased steric hindrance of the isopropyl group probably prevents a second reductive amination. *N*-isopropyl sophorolipid amine **214b** was obtained in high purity after reaction work-up and did not require further purification.



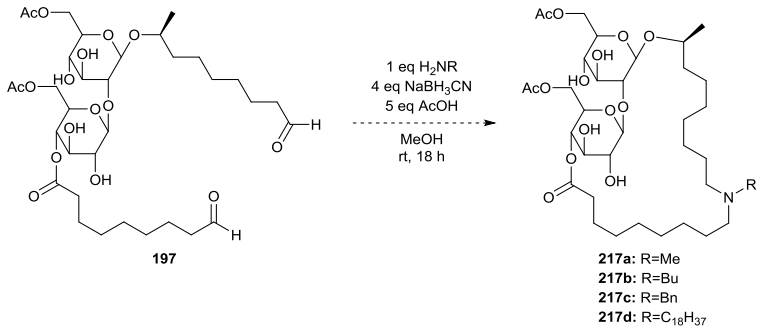
Scheme 62. Reductive amination towards *N*-isopropyl sophorolipid amine 214b

In the course of the development of the synthetic pathway towards sophorolipid aldehyde **201**, the reductive amination of sophorolipid dialdehyde **197** was also evaluated with primary amines (Scheme 63). When the reaction was performed with 2 equivalents of primary amine, no formation of the desired sophorolipid diamine **216** could be detected by LC-MS analysis. Instead, the formation of an amino-sophorolipid lactone **217** was observed, which is in accordance with the results obtained for the reductive amination of sophorolipid aldehyde **201** with primary amines.



Scheme 63. Reductive amination of sophorolipid dialdehyde 197 with primary amines

Natural sophorolipid lactones possess better antimicrobial and anticancer activities than the sophorolipid acid derivatives (*vide supra*).[46, 58] Therefore, it was attempted to selectively synthesize a varied set of amino-sophorolipid lactones **217** which could subsequently be quaternized in order to enhance their antimicrobial activity. According to the reductive amination of sophorolipid aldehyde **201** with primary amines, the selective synthesis of amino-sophorolipid lactones **217** was attempted with 1 equivalent of methylamine, butylamine, benzylamine or octadecylamine (Scheme 64). Although the formation of methyl, benzyl and octadecyl sophorolipid lactones **217a**, **217c** and **217d** was confirmed by LC-MS and NMR analysis, the purification of these derivatives *via* automated flash chromatography was not successful. An extensive evaluation of the purification of these derivatives and successful synthesis of butyl amino-sophorolipid lactone **217b** still has to be performed.

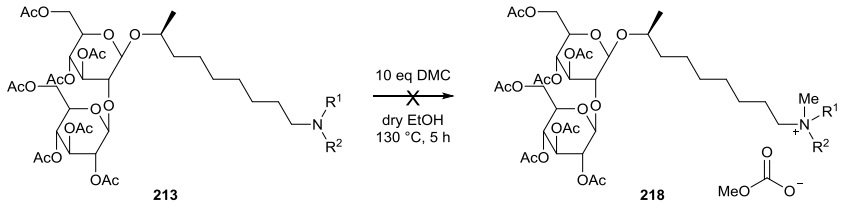


Scheme 64. Reductive amination of sophorolipid dialdehyde 197 towards amino-sophorolipid lactones 217

3.2.2. Synthesis of quaternary ammonium sophorolipids

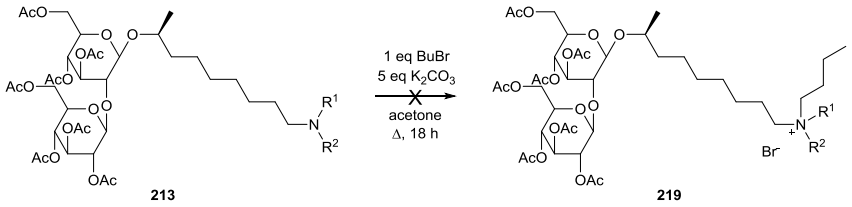
The sophorolipid tertiary amines **213** were used for the synthesis of a varied set of quaternary ammonium sophorolipids. Quaternization of the derivatives can have a great influence on the solubility and biological activity of the derivatives. For example, quaternary ammonium salts are a class of cationic surfactants which often possess antimicrobial activities. Moreover, several cationic lipids have proven to be efficient gene delivery vectors (*vide infra*).

At first, it was attempted to synthesize the methyl carbonate quaternary ammonium salts **218** *via* reaction with dimethyl carbonate (Scheme 65).[130] The reaction was performed in dry ethanol in a pressure vial with 10 equivalents of dimethyl carbonate. After evaporation of the solvent and excess of dimethyl carbonate, only the starting product could be detected by NMR-analysis.



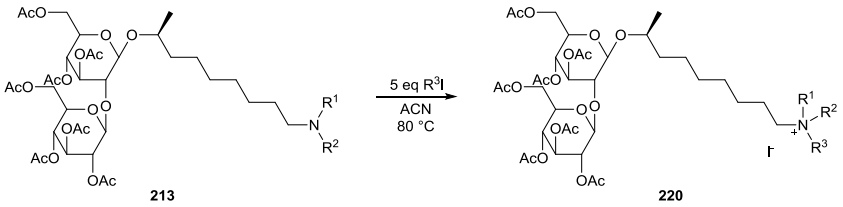
Scheme 65. Synthesis of methyl carbonate quaternary ammonium salts 218

The quaternization reaction was also evaluated with butyl bromide (Scheme 66). The reaction was performed in acetone with 1 equivalent of butyl bromide and 5 equivalents of potassium carbonate. Also with this procedure, only the starting product could be detected by NMR-analysis.



Scheme 66. Synthesis of bromide quaternary ammonium salts 219

Alternatively, alkyl iodides were applied for the quaternization reaction. The reaction was performed in acetonitrile at room temperature with addition of 2.6 equivalents of methyl or butyl iodide at 0 °C. Although quaternization did occur with this procedure, only partial conversion was obtained after 6 days. When the reaction was performed in a pressure vial at 80 °C with 5 equivalents of alkyl iodide, complete conversion could be obtained after 18 or 48 hours for quaternization with methyl or butyl iodide, respectively (Scheme 67). The quaternary ammonium sophorolipids **220** could be further purified *via* sonication of the product in ether. However, this purification was mostly not necessary. A set of nine different peracetylated quaternary ammonium sophorolipids **220** was synthesized (Table 5, Figure 11). Quaternization of *N*,*N*-dibenzyl sophorolipid amine **213g** with butyl iodide was also attempted, but this reaction was not successful.



Scheme 67. Synthesis of iodide quaternary ammonium salts 220

Table 5. Reaction conditions for the synthesis of quaternary ammonium sophorolipids 220

Amine	R^3I	Time (h)	Yield (%)	
213a	methyl iodide	18	**220a**	91
213c	methyl iodide	18	**220b**	89
213d	methyl iodide	18	**220c**	96
213d	butyl iodide	48	**220d**	94
213e	methyl iodide	18	**220e**	quant.
213f	methyl iodide	18	**220f**	89
213g	methyl iodide	18	**220g**	quant.
213h	methyl iodide	18	**220h**	98
213h	butyl iodide	48	**220i**	quant.

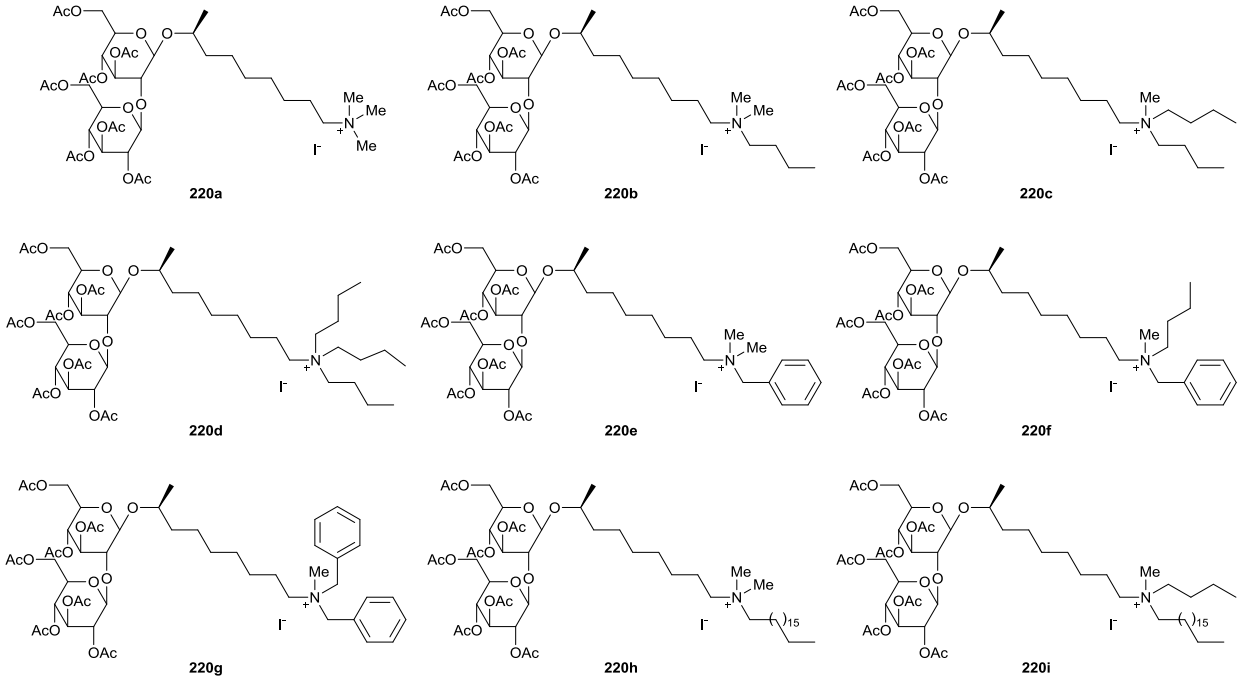
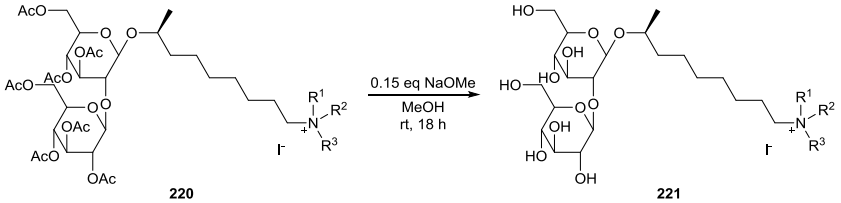


Figure 11. Library of peracetylated quaternary ammonium sophorolipids 220

This set of nine peracetylated quaternary ammonium sophorolipids was deprotected to obtain water soluble quaternary ammonium salt derivatives. This deprotection was performed *via* an alkaline methanolysis with 0.15 equivalents of sodium methoxide in methanol (Scheme 68). The quaternary ammonium sophorolipids **221** could be further purified *via* sonication in acetone. This also resulted in the synthesis of a set of nine different quaternary ammonium sophorolipids (Table 6, Figure 12).



Scheme 68. Deprotection *via* alkaline methanolysis towards quaternary ammonium sophorolipids 221

Table 6. Yield for the synthesis of quaternary ammonium sophorolipids 221

Quaternary ammonium sophorolipid	Yield (%)	
220a	**221a**	quant.
220b	**221b**	88
220c	**221c**	quant.
220d	**221d**	quant.
220e	**221e**	quant.
220f	**221f**	quant.
220g	**221g**	99
220h	**221h**	97
220i	**221i**	66

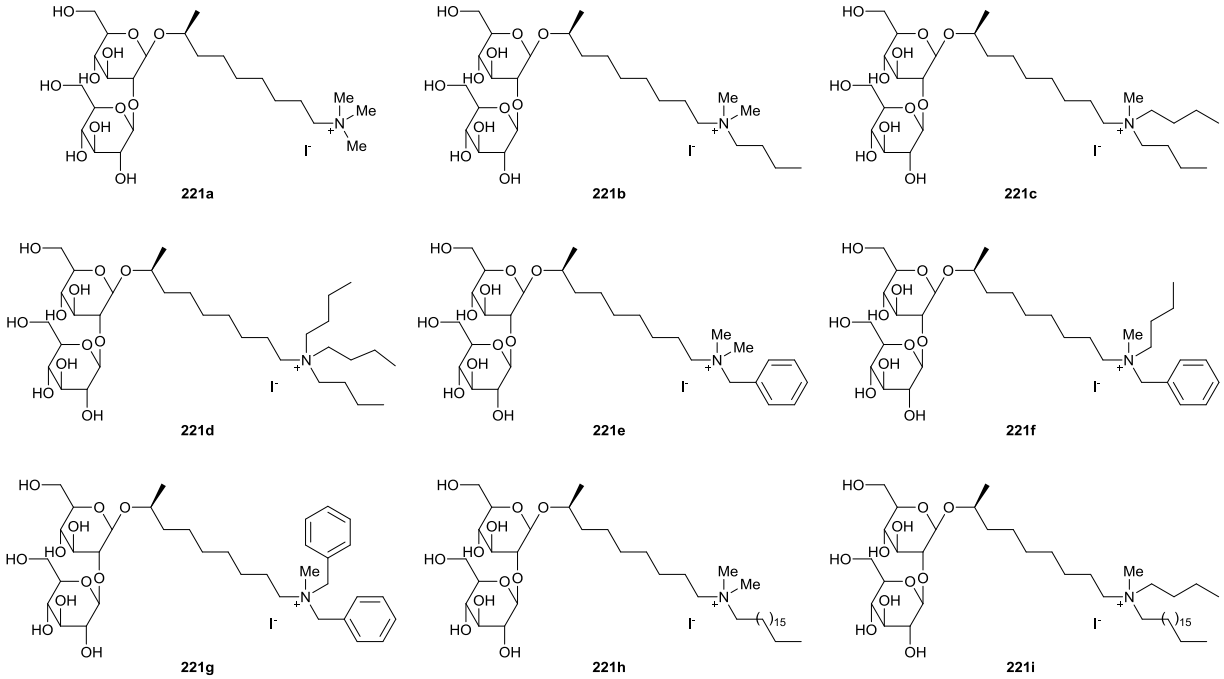


Figure 12. Library of deprotected quaternary ammonium sophorolipids 221

Measurement of the CMC value and the corresponding surface tension was attempted for the quaternary ammonium sophorolipids. The CMC of a surfactant is the lowest concentration for which micelles are formed. The surface tension measurements were determined in cooperation with the Particle and Interfacial Technology Group (Ghent University) *via* the Wilhelmy plate method (see Experimental procedures). The peracetylated quaternary ammonium sophorolipids **220** are not easily soluble in water. Sonication at elevated temperatures was necessary to dissolve the compounds at a concentration of 1 g/L. The measurements were performed with an intermediate washing step of the

platinum plate in a saturated sodium dodecyl sulfate solution. Without this washing step, it seemed that the platinum plate got contaminated with the product since measurement of pure water did no longer result in a surface tension of 72 mN/m. At a concentration of 1 g/L, the CMC was not reached for compounds **220a** and **220f** (Figure 13). Evaluation at higher concentrations was not performed due to the poor solubility of the compounds. The measurement of CMC values only makes sense when the compounds form a clear solution in water.

The deprotected sophorolipid quaternary ammonium salts **221** are readily soluble in water. However, the CMC was not reached at a concentration of 1 g/L for compounds **221a** and **221c** (Figure 14). For compound **221c**, extra measurements were performed starting at a concentration of 15.5 g/L. Even at this high concentration, the CMC was not reached. Evaluation at higher concentrations did not seem sensible in the light of surfactant properties. Surface tension measurements for compounds **221b** and **221h** resulted in decreasing surface tensions for decreasing concentrations which is not sensible at all. It was not possible to perform sensible measurements for these compounds. One hypothesis that could explain these unexpected results is that other types of nanostructures are formed, which are dependent on the concentration of the compound.

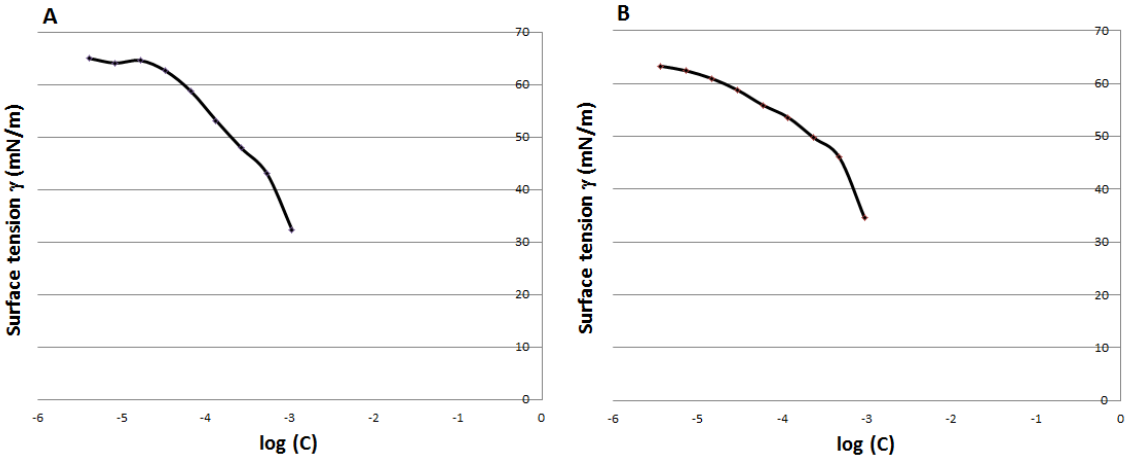


Figure 13. Surface tension measurements at different concentrations (logC) for compounds 220a (A) and 220f (B)

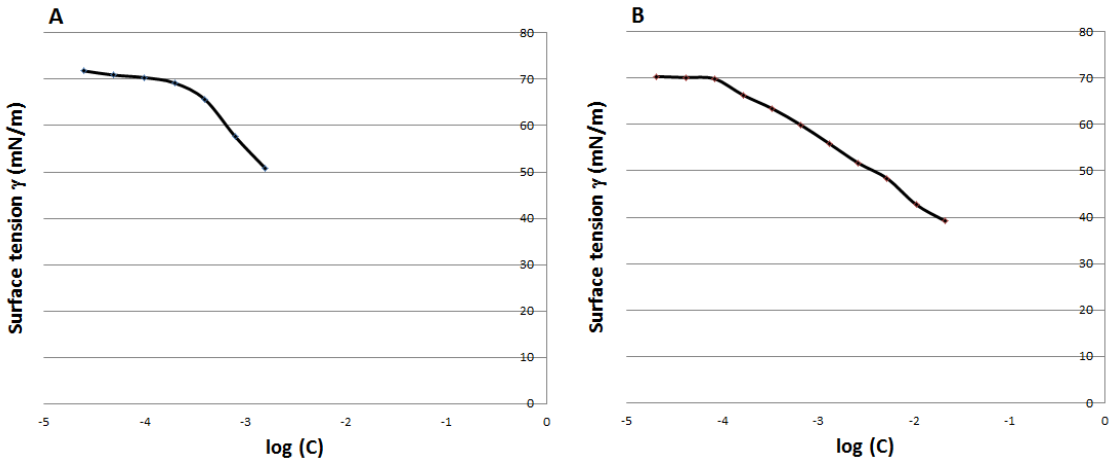


Figure 14. Surface tension measurements at different concentrations (logC) for compounds 221a (A) and 221c (B)

The self-assembly behavior of the quaternary ammonium sophorolipid **220** and **221** was evaluated *via* small-angle X-ray scattering (SAXS) analysis (see Experimental procedures). The evaluation of the self-assembly was performed by Dr. Niki Baccile with the help of Lisa Van Renterghem and Isabelle Van de Velde. All samples were analyzed in milliQ grade water at room temperature in a final concentration of 0.5 wt %. The samples were then analyzed within 1 to 3 hours after sample preparation. The peracetylated quaternary ammonium sophorolipids **220** required sonication and heating to dissolve them completely. These samples revealed to be sensitive to the X-ray beam and underwent beam damage. To overcome this problem, which was probably due to the presence of the acetyl groups, short acquisition times in combination with a flow-through analysis were used.

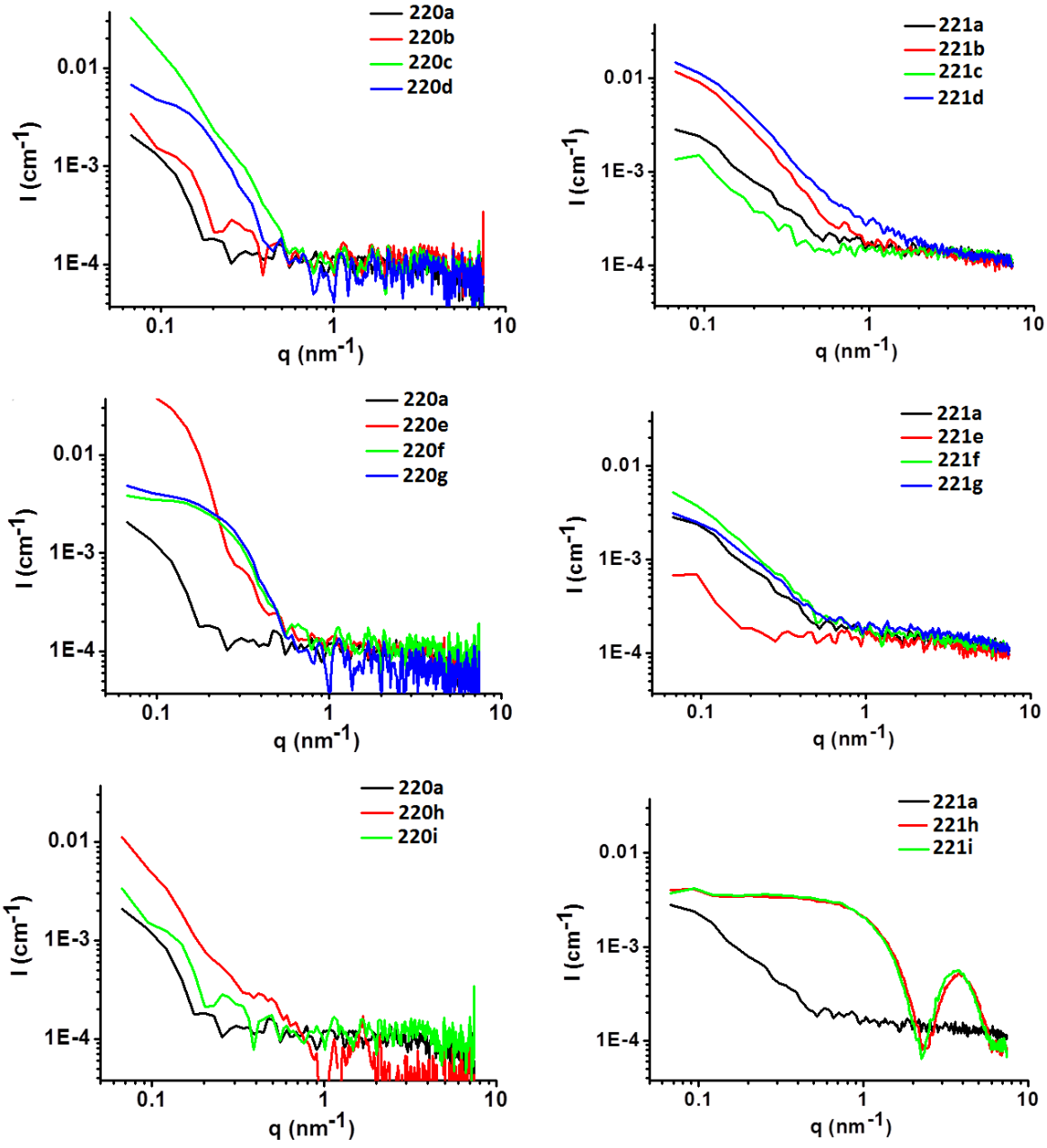


Figure 15. Small-angle X-ray scattering (SAXS) pattern with the q-value in function of the scattering intensity I

For almost all compounds, the curves display an intense scattering signal at $q < 0.7$ nm^{-1} (Figure 15). This pronounced scattering signal at low q-values indicates that large aggregates are formed. However, the amount of aggregates is probably quite low. At higher q-values, the signal is very low and noisy. The deprotected quaternary ammonium sophorolipids **221h** and **221i**, having an octadecyl chain on the nitrogen atom, display a scattering response which is very different from all other compounds. In this case, the signal shows a plateau at low q-values with a clear oscillation centered at a q-value around 4 nm^{-1}. This shape is typical for spherical micelles and the model-independent Guinier analysis indicates a radius of 3.3 ± 0.1 nm for both samples. This nice behavior may be attributed to the presence of the long aliphatic chain in the quaternary ammonium sophorolipids, which is much shorter for all other derivatives. It can be hypothesized that if the quaternary ammonium group is located close to the carbohydrate head, a long aliphatic chain will be needed to provide a sufficient hydrophobic tail for micelle formation. More experiments such as transmission electron microscopy (TEM) analysis are needed to further study the self-assembly behavior of the quaternary ammonium sophorolipids.

The antimicrobial activity of the quaternary ammonium sophorolipids **220** and **221** was evaluated together with sophorolipid aldehyde **201**, sophorolipid alcohol **204** and tertiary sophorolipid amines **213a-e/g-h**. The evaluation of the antimicrobial activities was performed by the Laboratory for Microbiology (Ghent University). The Gram-negative strains *Escherichia coli* LMG 8063 and *Klebsiella pneumoniae* LMG 2095, and the Gram-positive strains *Staphylococcus aureus* LMG 8064 and *Bacillus subtilis* LMG 13579 were chosen as test strains. The bioassay was carried out in 96-well microtiter plates at a concentration of approximately 0.5 mg/mL of the test compound and 10^4 CFU/mL test bacteria. None of the test compounds showed significant growth inhibition of *Escherichia coli* LMG 8063 and *Klebsiella pneumoniae* LMG 2095. Compounds **220b**, **220c**, **220d**, **220e**, **220f**, **220g**, **220h**, **220i**, **221b**, **221h** and **221i** showed significant growth inhibition of *Staphylococcus aureus* LMG 8064 and *Bacillus subtilis* LMG 13579.

For the active compounds, the minimum inhibitory concentration (MIC) was determined against a test panel of four Gram-positive strains, namely *Staphylococcus aureus* LMG 8064, *Enterococcus faecium* LMG 11397, *Bacillus subtilis* LMG 13579 and *Streptococcus pneumoniae* LMG 16738. The MIC value is considered as the lowest concentration of the test compound for which a lack of visible bacterial growth is observed. The bioassay was carried out in 96-well microtiter plates at a concentration range between 100 and 2.5 µg/mL or 1000 and 5 µg/mL of the test compounds for respectively strong or weak inhibitors and 10^4 CFU/mL test bacteria. The MIC values for the active compounds are given in Table 7 together with the MIC values for the antibiotic gentamicin sulfate.

Microscopic analysis in addition to the determination of the MIC values revealed that lysis of the cells occurred at the active concentrations.

The lowest MIC values of 5 μg/mL was obtained with compounds **221h** and **221i** against all four Gram-positive test strains. Low MIC values of 10 μg/mL were obtained with compounds **220h** and **220i**, also against all four Gram-positive test strains. Interestingly, these activities lie in the same concentration range as that of the antibiotic gentamicin sulfate. All four compounds perform as good or better as gentamicin sulfate against *E. faecium* and *S. pneumoniae*. Compounds **221h** and **221i** even perform as good as gentamicin sulfate against *S. aureus* and *B. subtilis*. For better comparison, the MIC values were converted on the basis of their molecular weight (Table 8). On this basis, we can conclude that compounds **220h**, **220i**, **221h** and **221i** are more active against all four Gram-positive test strains than the antibiotic gentamicin sulfate.

Gentamicin sulfate is used to treat several types of antibiotic infections caused by the Gram-negative strains *Pseudomonas aeruginosa*, *Proteus* species, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens* and the Gram-positive meticillin-susceptible *Staphylococcus* species.[131] This antibiotic is included by the World Health Organization (WHO) in the List of Essential Medicines.[132] Its mode of action is binding to the 30S subunit of bacterial ribosomes, hereby preventing the protein synthesis. Gentamicin sulfate also binds to the lipopolysaccharide layer of Gram-negative bacteria, hereby disrupting the permeability of the cell wall. Although the quaternary ammonium sophorolipids **220h** and **220i** are not active against Gram-negative strains, their activity against Gram-positive strains can be considered as reasonably good. However, the fact that gentamicin sulfate is mostly active against Gram-negative strains makes it difficult to give an appreciation of the activity of the quaternary ammonium sophorolipids **220h** and **220i** in comparison with this antibiotic. Moreover, it should be taken into account that these results are obtained with *in vitro* testing and that further *in vivo* testing is necessary to determine the actual antibiotic potential of these compounds. It would have been more informative to include β-lactam antibiotics as reference compounds since these are mostly active against Gram-positive strains. Their mode of action is inhibiting the synthesis of the peptidoglycan layer in the bacterial cell wall, resulting in cytolysis and cell death. Quaternary ammonium compounds are known to exert antibiotic properties. Their activity is related to their cationic surfactant properties and their mode of action is interacting with the cell membrane, resulting in cell damage and leaking.[133-134] Microscopic analysis revealed that cell lysis occurred at the active concentrations, which supports both hypotheses. However, since these sophorolipid derivatives are quaternary ammonium compounds, the second hypothesis seems most plausible. More experiments are needed, for example with isotopic labeled derivatives, to determine the mode of action.

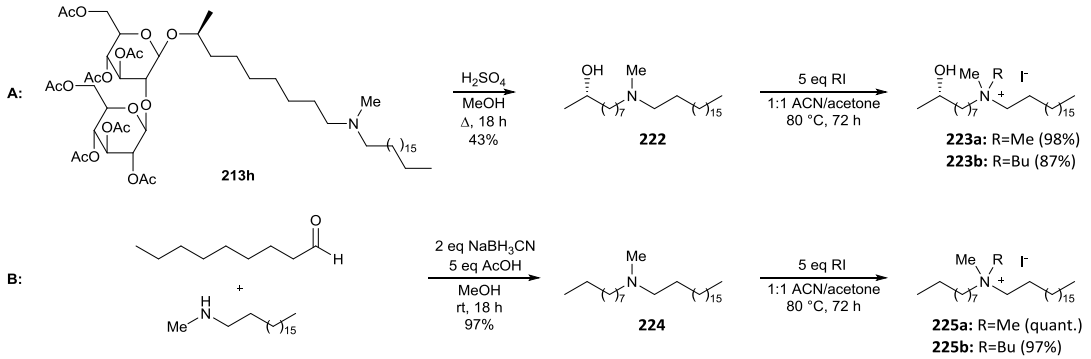
Table 7. Minimum inhibitory concentration (μg/mL) for the active compounds and the antibiotic gentamicin sulfate

	220b	**220c**	**220d**	**220e**	**220f**	**220g**	**220h**	**220i**	**221b**	**221h**	**221i**	Gentamicin sulfate
S. aureus	>100	>100	>100	500	>100	50	10	10	>100	5	5	5
E. faecium	>100	>100	>100	>1000	>100	>100	10	10	>100	5	5	10
B. subtilis	>100	25	>100	1000	>100	50	10	10	>100	5	5	5
S. pneumoniae	>100	100	>100	1000	>100	100	10	10	>100	5	5	25

Table 8. Minimum inhibitory concentration (μM) for the active compounds and the antibiotic gentamicin sulfate

	220b	**220c**	**220d**	**220e**	**220f**	**220g**	**220h**	**220i**	**221b**	**221h**	**221i**	Gentamicin sulfate
S. aureus	>101	>97	>93	489	>94	45	8	8	>144	6	5	10
E. faecium	>101	>97	>93	>977	>94	>91	8	8	>144	6	5	21
B. subtilis	>101	24	>93	977	>94	45	8	8	>144	6	5	10
S. pneumoniae	>101	97	>93	977	>94	91	8	8	>144	6	5	52

To evaluate the influence of the carbohydrate head on the antimicrobial properties, the deglycosylated derivatives of quaternary ammonium sophorolipids **220h** and **221i** were also synthesized. In a first step, the synthesis of hydroxylated quaternary ammonium salts **223** was attempted starting from *N*-methyl,*N*-octadecyl sophorolipid amine **213h** (Scheme 69A). The sophorolipid amine was subjected to acid methanolysis, followed by purification *via* automated column chromatography. (*S*)-9-(methyl(octadecyl)amino)nonan-2-ol **222** was subsequently quaternized with methyl and butyl iodide towards the hydroxylated quaternary ammonium salts **223a** and **223b**. These compounds possess a subterminal hydroxyl function, resulting from the stereoselective hydroxylation of the fatty acid in the fermentation process. Since this hydroxyl function could also influence the amphiphilic properties of the quaternary ammonium salts, synthesis of the non-hydroxylated quaternary ammonium salts **225** was attempted as well (Scheme 69B). *N*-methyl,*N*-nonyloctadecan-1-amine **224** was synthesized *via* reductive amination of nonanal and *N*-methyl,*N*-octadecylamine, followed by quaternization with methyl and butyl iodide towards the non-hydroxylated quaternary ammonium salts **225a** and **225b**.



Scheme 69. Synthesis of hydroxylated and non-hydroxylated quaternary ammonium salts 223 and 225

The antimicrobial activity of the deglycosylated quaternary ammonium salts **223** and **225** was evaluated together with the quaternary ammonium sophorolipids **220h-i** and **221h-i** and the natural diacetylated sophorolipid lactone **1** and sophorolipid acid **2**. The evaluation of the antimicrobial activities was performed by the Laboratory of Pharmaceutical Microbiology (Prof. T. Coenye, Ghent University). The Gram-negative bacteria *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095 and *Pseudomonas aeuruginosa* PAO1, and the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 (a multi-resistent strain) were chosen as test organisms. The bioassay was carried out in 96-well plates in a concentration series ranging from 1000 to 0.48 µg/mL of test compound and approximately 5 x 10^4 bacteria in a final volume of 200 µL. Only one of the compounds showed weak activity against one of the Gram-negative strains, *i.e.* quaternary ammonium sophorolipid **221i** displayed a $MIC_{1/2}$ of 500 µg/mL against *P. aeruginosa*. $MIC_{1/2}$ is the minimum inhibitory concentration at which the growth of the strain is reduced by 50%.

For all ten compounds, both MIC and minimum bactericidal concentration (MBC) values were determined against the two Gram-positive strains (Table 9). The MBC value is considered as the lowest concentration of the test compound at which no more viability of the test organism can be observed. Therefore, it is an indication of the microbial death whereas the MIC value is only an indication of the inhibition of microbial growth. The MIC values obtained for the quaternary ammonium sophorolipids **220h-i** and **221h-i** against *S. aureus* ATCC 6538 were consistent with the previously obtained results.

Table 9. Minimum inhibitory and bactericidal concentrations (µg/mL) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

		1	**2**	**220h**	**220i**	**221h**	**221i**	**223a**	**223b**	**225a**	**225b**
***S. aureus* ATCC 6538**	**MIC**	31.25	>1000	7.81	7.81	1.95	1.95	1.95	1.95	31.25	15.63
	MBC	62.5	>1000	7.81	250	7.81	1.95	31.25	31.25	31.25	15.63
***S. aureus* Mu50**	**MIC**	62.5	>1000	31.25	62.5	3.9	3.9	62.5	62.5	125	62.5
	MBC	62.5	>1000	62.5	250	15.63	15.63	62.5	62.5	125	125

The best results were obtained for the deprotected quaternary ammonium sophorolipid **221h** and **221i** against both *S. aureus* strains, especially when compared on basis of their molecular weight (Table 10). This clearly indicates that the presence of the carbohydrate head has a positive effect on the antimicrobial activity. Moreover, the hydroxylated quaternary ammonium salts **223** perform generally better than the non-hydroxylated quaternary ammonium salts **225**. In view of these results, it can be hypothesized that an increased hydrophilic character of the compounds results in an increased antimicrobial activity. However, no conclusions on the mode of action can be made based on these data.

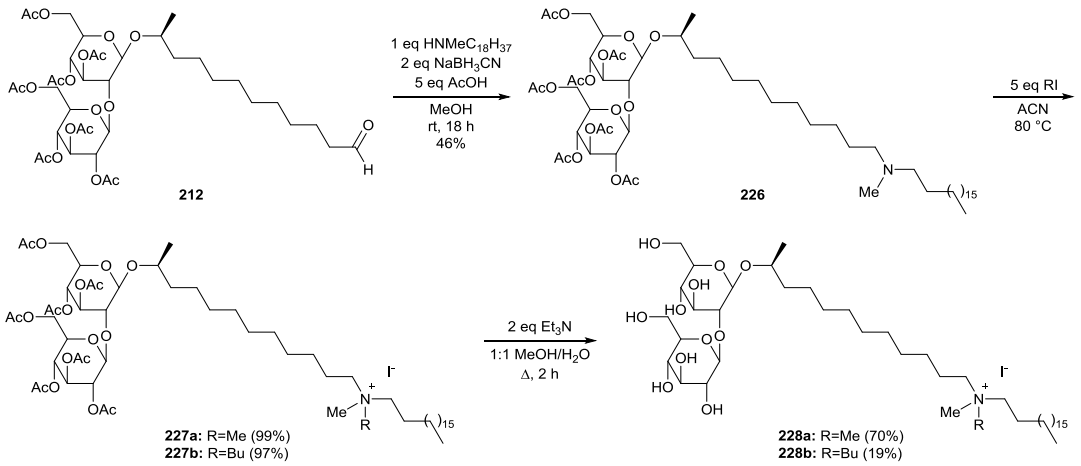
Table 10. Minimum inhibitory and bactericidal concentrations (µM) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

		1	**2**	**220h**	**220i**	**221h**	**221i**	**223a**	**223b**	**225a**	**225b**
***S. aureus* ATCC 6538**	**MIC**	45.4	>1607	6.59	6.36	2.18	2.09	3.44	3.21	56.68	26.33
	MBC	90.79	>1607	6.59	204	8.76	2.09	55.08	51.28	56.68	26.33
***S. aureus* Mu50**	**MIC**	90.79	>1607	26.36	50.91	4.37	4.18	110	103	227	105
	MBC	90.79	>1607	52.72	204	17.53	16.73	110	103	227	211

The deprotected quaternary ammonium sophorolipids **221h** and **221i** were evaluated on their ability to affect an already established biofilm of *S. aureus* ATCC 6538 and *S. aureus* Mu50. This biofilm assay was performed in 96-well plates with previously formed biofilms after removal of the non-adhered cells. At a concentration of 20 µg/mL of test compound, no effect was observed for both compounds against both *S. aureus* strains. This concentration was higher than both the MIC and MBC value of both compounds.

S. aureus Mu50 is a meticillin-resistant *Staphylococcus aureus* (MRSA) strain with vancomycin resistance. Therefore, the activities obtained with the quaternary ammonium sophorolipids **221h** and **221i** against both *S. aureus* strains can be considered as reasonably good. However, it should be taken into account that these results are obtained with *in vitro* testing and that further *in vivo* testing is necessary to determine to actual antibiotic potential of these compounds.

In view of the good antimicrobial activities obtained for the C9 quaternary ammonium sophorolipids which possess an octadecyl chain, the synthesis of their C12 counterparts was attempted (Scheme 70). The C12 *N*-methyl,*N*-octadecyl sophorolipid amine **226** was synthesized *via* reductive amination of C12 sophorolipid aldehyde **212** with *N*-methyl,*N*-octadecylamine. Sophorolipid amine **226** was purified *via* automated column chromatography with an ethyl acetate/triethylamine/hexane mixture as eluent. The subsequent quaternization was performed with methyl and butyl iodide for 18 and 48 hours, respectively, furnishing the corresponding peracetylated quaternary ammonium sophorolipids **227** in high purity. Deprotection was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions.[135] Evaporation of the reagent, solvent and methyl acetate byproduct yielded pure C12 quaternary ammonium sophorolipids **228**.



Scheme 70. Synthesis of C12 quaternary ammonium sophorolipids starting from sophorolipid aldehyde 212

The antimicrobial activity of the peracetylated C12 quaternary ammonium sophorolipids **227** and the deprotected C12 quaternary ammonium sophorolipids **228** was evaluated together with the petroselinic acid based sophorolipid lactone **208** and sophorolipid acid **209**. The evaluation of the antimicrobial activities was performed by the Laboratory of Pharmaceutical Microbiology (Prof. T. Coenye, Ghent University). The Gram-negative bacteria *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095 and *Pseudomonas aeruginosa* PAO1, and the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 (a multi-resistant strain) were chosen as test organisms. The bioassay was performed as described for the deglycosylated derivatives **223** and **225** (*vide supra*).

Table 11. Minimum inhibitory concentrations (µg/mL) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

	208	209	227a	227b	228a	228b
***S. aureus* ATCC 6538**	31.25	>1000	15.63	15.63	62.5	31.25
***S. aureus* Mu50**	250	>1000	1000	500	62.5	62.5

Only one of the compounds, i.e. deprotected quaternary ammonium sophorolipid **228a**, showed weak activity against the two Gram-negative strains *K. pneumoniae* and *P. aeruginosa* with a MIC value of 1000 µg/mL against both organisms. For the petroselinic acid based sophorolipid lactone **208** and sophorolipid acid **209**, similar activities were obtained as their oleic acid based counterparts (Table 11). Only the activity of petroselinic acid based sophorolipid lactone **208** against *S. aureus* Mu50 is considerably lower than the activity of oleic acid based sophorolipid lactone **1**. The activity of all four C12 quaternary ammonium sophorolipids is lower than their corresponding C9 quaternary ammonium sophorolipids, even when compared on a molecular basis (Table 12). Whereas the MIC values of the peracetylated quaternary ammonium sophorolipids **227** against *S. aureus* ATCC 6538 still lie in the same concentration range as those of the corresponding C9 compounds, their MIC values against *S. aureus* Mu50 are much higher. The MIC values of the deprotected quaternary ammonium sophorolipids **228** against both *S. aureus* strains are also considerably higher than those of the corresponding C9 compounds.

Table 12. Minimum inhibitory concentrations (µM) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

	208	209	227a	227b	228a	228b
***S. aureus* ATCC 6538**	45.40	>1607	12.73	12.31	66.9	32.03
***S. aureus* Mu50**	363.2	>1607	815	394	66.9	64.1

The quaternary ammonium sophorolipids **220** and **221** and the deglycosylated quaternary ammonium salts **223** and **225** were also evaluated on their suitability as gene delivery vectors. These transfection efficacies were determined by the CEMCA UMR 6521 and INSERM UMR 1078 research groups (Prof. P.-A. Jaffrès, Brest University). Cationic lipids constitute a class of amphiphilic compounds which can be used to compact, protect and carry pDNA or other nucleic acids such as mRNA, shRNA or siRNA for *in vitro* or *in vivo* applications.[136-140] This class of vectors was initially used by Felgner *et al*.[141] Since then, many types of cationic lipids were designed, leading to a better understanding of the mode of action and the identification of efficient vectors.[142-144] For example, recent clinical trials demonstrated some benefits for the administration of CFTR genes to cystic fibrosis patients using a cationic lipid as carrier.[145] Moreover, synthetic vectors such as cationic lipids can be administered multiple times without any side reaction, in contrast to viral vectors which are efficient carriers for transfection but can induce an immune response from the first administration.[146-147] Many cationic lipids possess a structure inspired on natural amphiphilic

compounds such as phospholipids with the aim to produce non-toxic or low-toxic vectors.[148] Recently, research is focusing on the development of renewable based gene delivery vectors, since natural derived products are expected to have an enhanced biocompatibility.[149-162] Glycerol-based cationic lipids were widely studied and cationic lipids with natural lipid chains such as oleyl, linoleyl or phytanyl chains were used to produce efficient vectors.[149-152] MacDonald and co-workers alkylated natural diacylglycerophosphocholine to produce cationic lipids.[153-155] Aminoglycosides, spermine-based vectors and trimethylarsonium-based compounds (these latter being widely present in sea food) were also incorporated as cationic polar head to produce efficient vectors for gene delivery.[139, 156-164] Therefore, the assessment of new natural cationic amphiphilic compounds for nucleic acid delivery is of great interest. In view of the multiple beneficial biological activities and self-assembly properties described for the natural sophorolipids (*vide supra*), cationic sophorolipid derivatives can be considered as suitable vectors for gene delivery.

From the set of eighteen quaternary ammonium sophorolipids, five compounds (**220a**, **220h**, **221a**, **221h** and **221i**) were evaluated for their ability to form supramolecular aggregates in water solution by using the method of hydration of a lipid film. In order to determine the importance of the carbohydrate head, the deglycosylated quaternary ammonium salts **223** and **225** were also evaluated. For all liposomal solutions, the size of the particles and their surface charge were determined *via* DLS and zeta measurements, respectively. The formation of a homogenous formulation is a necessary prerequisite for the evaluation of the suitability of quaternary ammonium salts as vectors for gene delivery. From the five compounds which were evaluated, only compounds **221h** and **221i** produced homogenous formulations. For the other quaternary ammonium sophorolipids, their weak amphiphilic character (limited hydrophobic domain) likely explained the absence of well characterized nanoparticles in water. These results are consistent with the results obtained *via* the SAXS analysis. Compounds **221h**, **221i**, **223a/b** and **225a/b** were formulated with or without 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine (DOPE). Especially for the quaternary ammonium sophorolipids **221h** and **221i**, and to a lesser extent for hydroxylated quaternary ammonium salts **223a/b**, formulation with DOPE proved to be necessary to obtain homogenous formulations (Table 13). Formulations with DOPE also resulted in the formation of much smaller particles in comparison to formulations without DOPE. All zeta potentials were clearly positive as expected for liposomes generated from cationic lipid derivatives.

Table 13. Size and zeta potential measurements of liposomal solutions prepared at 1.5 mM without (left) or with (right) DOPE

	Size (nm)	Polydisp. index	Zeta (mV)	Size (nm)	Polydisp. index	Zeta (mV)
				+ DOPE		
221h	186 ± 110	0.94	29.9	54 ± 0.5	0.22	49.2
221i	275 ± 49	0.56	27.9	94 ± 0.1	0.20	40.8
223a	288 ± 23	0.53	35.9	35 ± 0.7	0.36	49.1
223b	289 ± 9	0.30	27.6	43 ± 0.8	0.28	49.4
225a	169 ± 2	0.34	42.4	47 ± 0.9	0.40	51.0
225b	212 ± 5	0.37	24.7	78 ± 1.2	0.27	52.9

The capacity to compact plasmid DNA (pDNA) was evaluated for all liposomal formulations by pDNA retardation assays on agarose gel electrophoresis. This was performed at different charge ratios (CR) which is defined as the number of positive charges provided by the cationic lipid derivative divided by the number of negative charges carried by the pDNA. A commercial lipofection agent, lipofectamine (LFM), and the cationic lipophosphoramidate BSV36 were used as reference compounds. In the absence of any co-lipid, no pDNA compaction was observed for any of the six compounds (Figure 16). When formulated with DOPE, quaternary ammonium sophorolipid **221h** and **221i** still remained almost inefficient to compact pDNA (Figure 17). For hydroxylated quaternary ammonium salts **223a/b**, very weak compaction was observed. Only both non-hydroxylated quaternary ammonium salts **225a/b** demonstrated some ability to compact pDNA, at a similar level as LFM. Altogether, these results suggest that a better compaction of pDNA is obtained for less hydrophilic compounds.

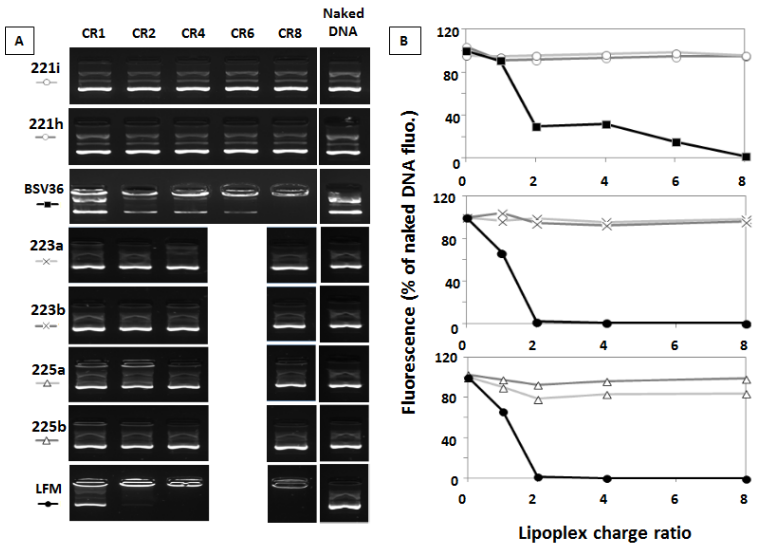


Figure 16. Ability for pDNA complexation of compounds 221h, 221i, 223a/b and 225a/b, formulated without DOPE, at various charge ratios (CR). For each compound, the profile of retardation assay (panel A) and the corresponding relative fluorescence intensity (panel B) of the lower DNA band (i.e. the supercoiled pDNA) are shown. The legend for panel B is given for each compound in panel A.

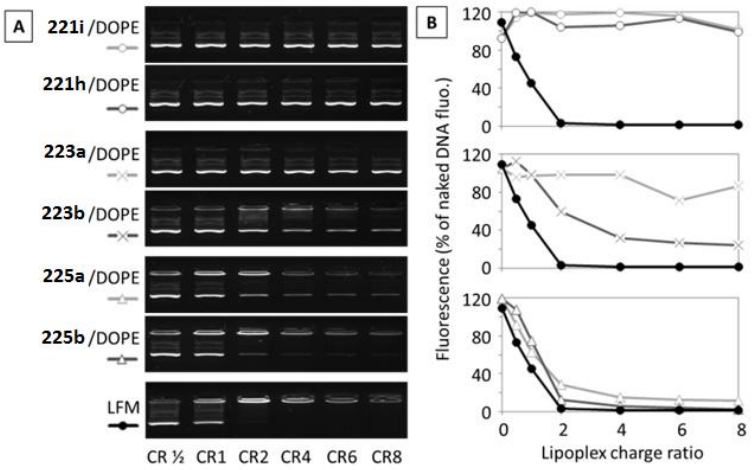


Figure 17. Ability for pDNA complexation of compounds 221h, 221i, 223a/b and 225a/b, formulated with DOPE, at various charge ratios (CR). For each compound, the profile of retardation assay (panel A) and the corresponding relative fluorescence intensity (panel B) of the lower DNA band (i.e. the supercoiled pDNA) are shown. The legend for panel B is given for each compound in panel A.

The cationic lipids where then evaluated in respect of their ability to deliver DNA into various cell lines. Three human-derived cell lines were included, namely melanoma cells (SKMEL28) and two airway epithelial cells, i.e. lung carcinoma (A549) and normal bronchial (16HBE) cells. A fourth cell line was a mouse myoblast cell line (C2C12) which was only used for the evaluation of the formulations incorporating DOPE. A reporter (luciferase-encoding) pDNA was used that allowed the determination of the transfection efficiency *via* highly sensitive luminescence measurements. Each formulation was evaluated at different CR, similar to those previously considered for the DNA complexation assay.

Concerning the efficiency of liposomes prepared without DOPE, only the ones derived from non-hydroxylated quaternary ammonium salts **225a/b** were able to transfect one of the cell lines studied (16HBE) (Figure 18). All other liposomes without DOPE were completely inefficient to transfect any of the considered cell lines. On the contrary, when formulated with DOPE, all derivatives demonstrated some ability to transfect one or more cell lines. The quaternary ammonium sophorolipids **221h** and **221i** efficiently transfected 16HBE and A549 cell lines, whereas lower efficiencies were obtained for the transfection of SKMEL28 cell line (Figure 19). This observation could be related to the low compaction properties of the formulations of compounds **221h** and **221i** with DOPE as shown in Figure 19. Two hypotheses can be formulated: 1) Compared to cell lines A549 and 16HBE, cell line SKMEL28 requires efficient pDNA compacting agents to observe transfection that likely occurs via an endocytosis pathway. This hypothesis is consistent with the compaction properties of **225a/b** or LFM and their respective transfection efficacies. 2) The second hypothesis would implicate a different mechanism that could involve the temporary poration of the membrane which was recently observed by Ilies and coworkers for another kind of cationic lipids.[165] The quaternary ammonium

salts **223a/b** and **225a/b** were also efficient to deliver the pDNA into all four cell lines with the best results obtained at CR2 or CR4 (Figure 20). It can be noticed that the chemical structure of **225a/b** is close to that of didodecyldimethylammonium bromide (DDAB) or dodecyltrimethylammonium bromide which were reported previously as vectors for gene delivery.[166-167]

Regarding the toxicity of the different compounds, it is noteworthy that they exhibited quite different effects towards the viability of the cells. However, these effects were similar whether or not DOPE was incorporated in the formulation (Figrue 21). Quaternary ammonium salts **223a/b** and **225a/b** were highly detrimental for the cells from a CR as low as 2 (Figure 22, Figure 23). This high toxicity was clearly associated with a decrease of the transfection efficacy at CR higher than 2, except for the C2C12 cell line where a decrease of the transfection efficacy occurred at CR higher than 4. This may be explained by a detergent effect exerted by these cationic amphiphilic compounds. On the contrary, quaternary ammonium sophorolipids **221h** and **221i** were much better tolerated by the cells. No real toxicity against SKMEL28 and only a moderate CR-dependent toxicity against the three other cell lines were noticed. These results strongly suggest that the biocompatibility of sophorolipids is – at least in part – due to the sugar group they specifically incorporate. Hence, despite that quaternary ammonium sophorolipids **221h** and **221i** poorly interacted with pDNA, they were clearly more efficient transfection vectors than their deglycosylated analogues **223a/b** and **225a/b** since they exert a much lower toxicity to the different cell lines. Many recent reports highlight that there is no strict relationship between the ability of a given vector to retard DNA on agarose gel and its ability to transfect cells *in vitro*. Actually, a balance should be found between stability of complexes (in order to form aggregates dense enough to contact and enter cells) and lability (to release DNA once inside the cells).[151, 168-169] However, no general conclusion can be drawn since such an equilibrium might depend on many parameters including the cationic lipid considered, the experimental conditions employed (e.g. the medium used to prepare the lipoplexes), the cells to treat, *etc.* Such a balance may also determine the toxicity experienced by the cells.

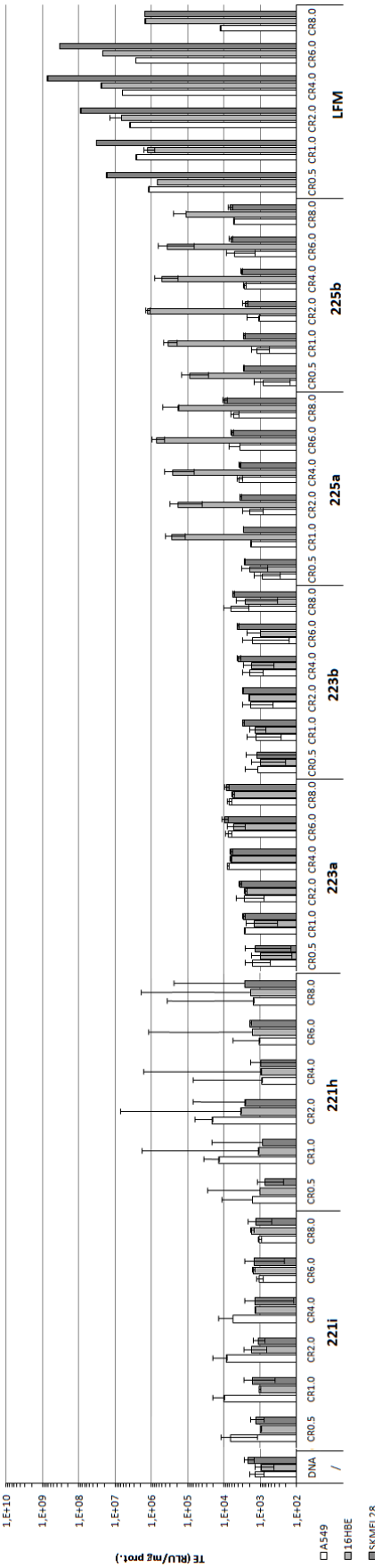


Figure 18. Transfection efficacies (TE) of compounds 221h, 221i, 223a/b and 225a/b, all formulated without DOPE, on three cell lines (A549, 16HBE and SKMEL28) using luciferase-encoding pDNA. TE are expressed in RLU/mg of proteins (n=3) (RLU=Relative light units). Lipofectamine (LFM) and naked (uncomplexed) pDNA were used as positive and negative control, respectively.

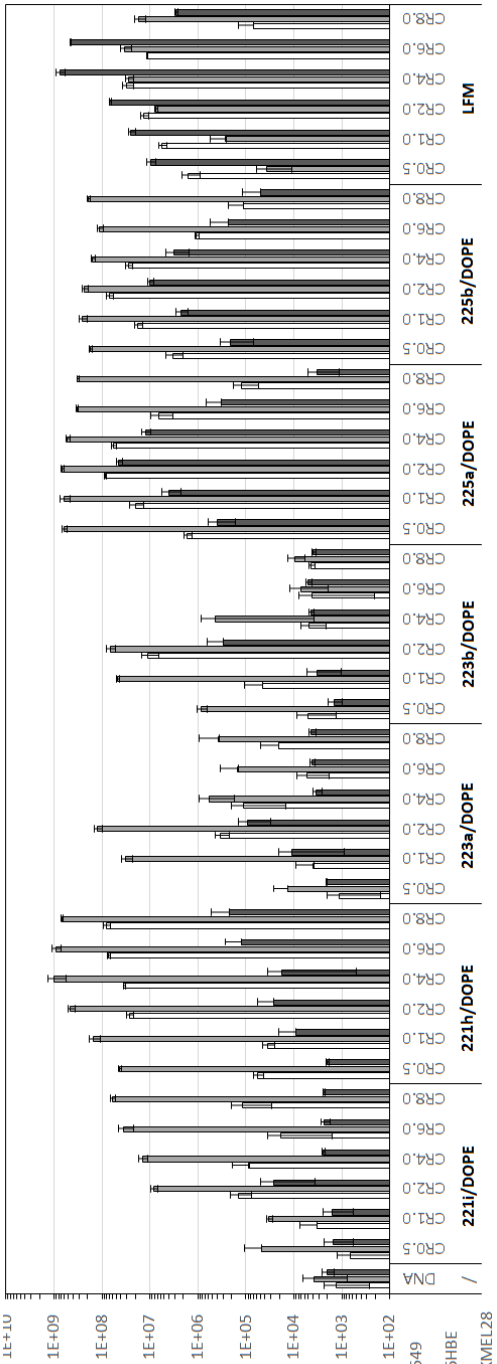


Figure 19. Transfection efficacies (TE) of compounds 221h, 221i, 223a/b and 225a/b, all formulated with DOPE, on three cell lines (A549, 16HBE and SKMEL28) using luciferase-encoding pDNA. TE are expressed in RLU/mg of proteins (n=3) (RLU=Relative light units). Lipofectamine (LFM) and naked (uncomplexed) pDNA were used as positive and negative control, respectively.

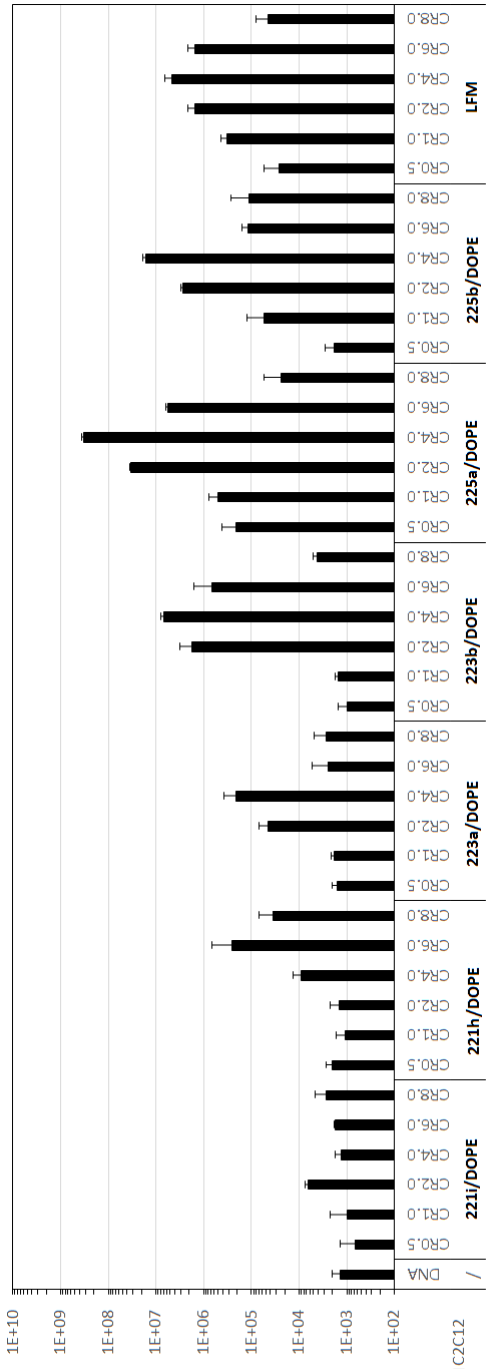


Figure 20. Transfection efficacies (TE) of compounds 221h, 221i, 223a/b and 225a/b, all formulated with DOPE, on a mouse myoblast cell line (C2C12) using luciferase-encoding pDNA. TE are expressed in RLU/mg of proteins (n=3) (RLU=Relative light units). Lipofectamine (LFM) and naked (uncomplexed) pDNA were used as positive and negative control, respectively.

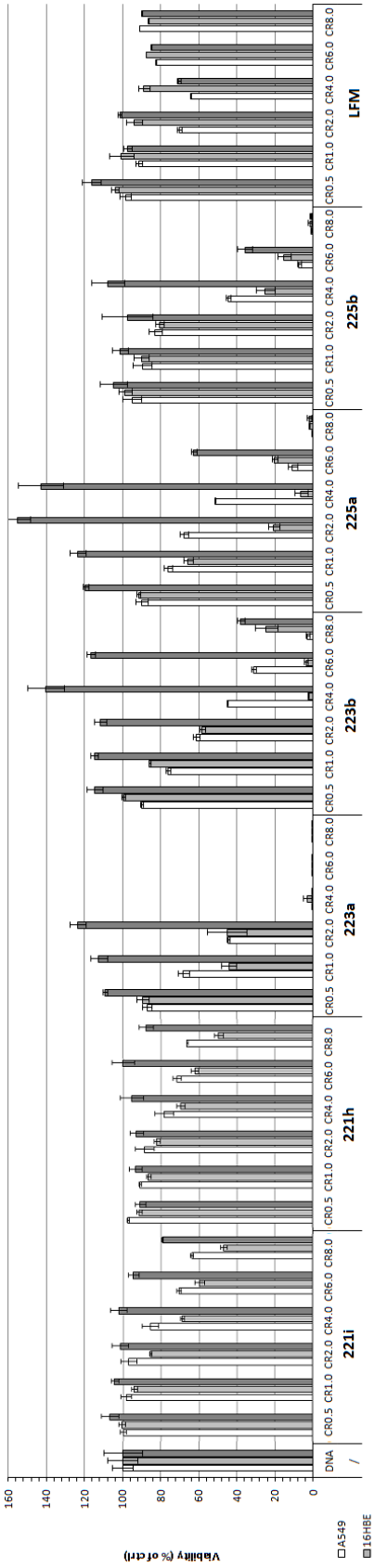


Figure 21. Cell viability of three cell lines (A549, 16HBE and SKMEL28) determined 48 hours after incubation of the cells with lipoplexes prepared with compounds 221h, 221i, 223a/b and 225a/b, all formulated without DOPE. Naked pDNA was used as negative control. Values are expressed as a percentage of the viability determined with untransfected cells.

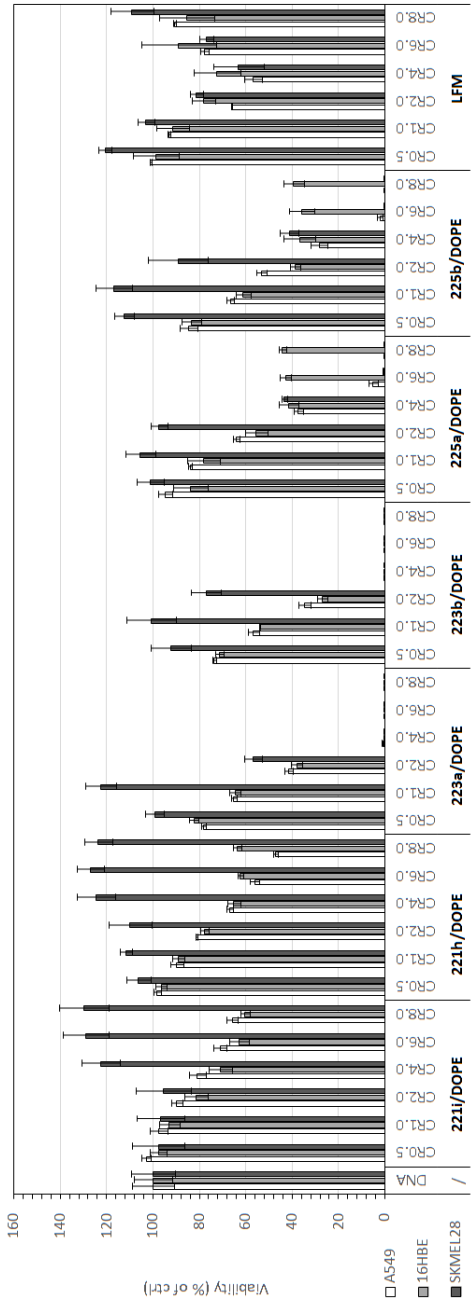


Figure 22. Cell viability of three cell lines (A549, 16HBE and SKMEL28) determined 48 hours after incubation of the cells with lipoplexes prepared with compounds 221h, 221i, 223a/b and 225a/b, all formulated with DOPE. Naked pDNA was used as negative control. Values are expressed as a percentage of the viability determined with untransfected cells.

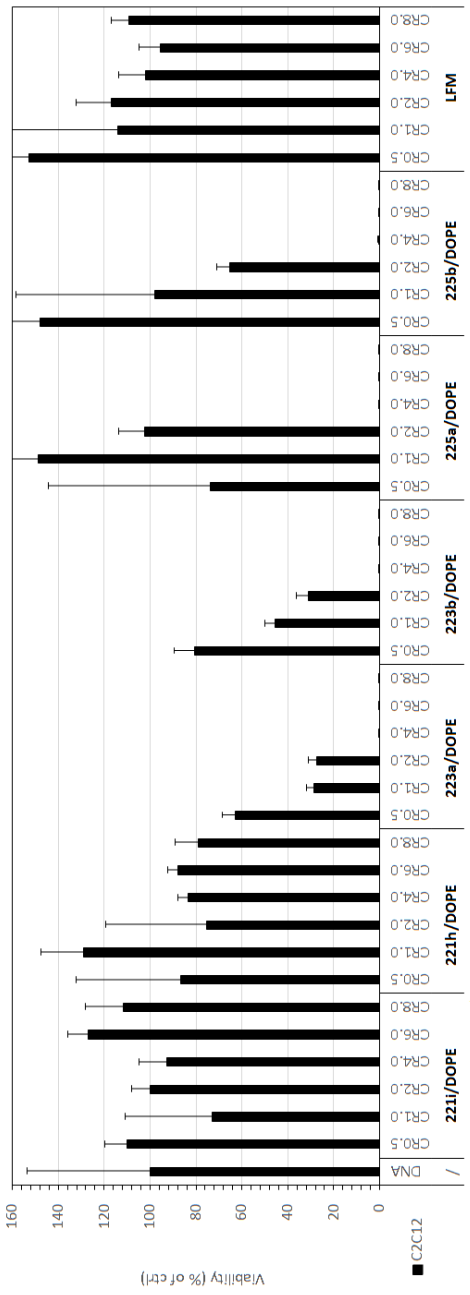


Figure 23. Cell viability of the mouse myoblast cell line (C2C12) determined 48 hours after incubation of the cells with lipoplexes prepared with compounds 221h, 221i, 223a/b and 225a/b, all formulated with DOPE. Naked pDNA was used as negative control. Values are expressed as a percentage of the viability determined with untransfected cells.

Different green chemistry metrics were also evaluated for the quaternary ammonium sophorolipids **221h** and **221i**, which proved to be the most promising derivatives of this chapter (Table 14). For both compounds, the valorization of the methyl 9-oxononanoate by-product is taken into account. The three extra reaction steps for the synthesis of the quaternary ammonium sophorolipids from sophorolipid aldehyde **201** result in a fourfold increase of the E-factor. However, these values lie between the range of E-factors for fine chemicals (5-50) and pharmaceuticals (25-100).

Table 14. Overview of the green chemistry metrics for the production of quaternary ammonium sophorolipids 221h and 221i. CE = carbon efficiency, AE = atom economy, SF = stoichiometric factor, RME = reaction mass efficiency, ε = reaction yield, c = mass reaction catalyst, s = mass reaction solvent, w = mass reaction waste, m = mass target product.

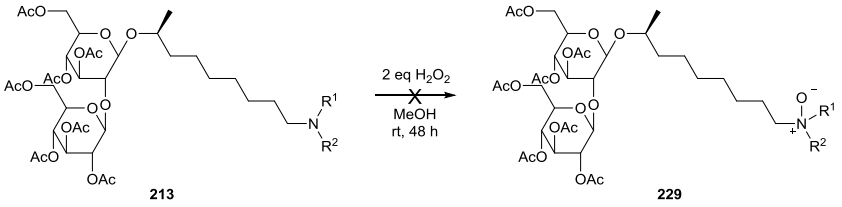
Parameter	Formula	221h	221i
CE (%)	$\frac{(\text{Amount of carbon in product}) * 100}{\text{Total amount of carbon in reagents}}$	60	61
AE (%)	$\frac{(\text{Molecular weigth of product}) * 100}{\sum \text{Molecular weigth of reagents}}$	55	56
SF	$1 + \frac{(\text{AE}) * \sum \text{mass of excess reagent}}{\text{Expected product mass at 100\% yield}}$	1.35	1.44
RME (%)	$\varepsilon * (\text{AE}) * \frac{1}{\text{SF}} * \left[\frac{1}{1 + \frac{\varepsilon * (\text{AE}) * (\text{c} + \text{s} + \text{w})}{(\text{SF}) * \text{m}}}\right]$	0.021	0.016
E-factor (kg/kg)	$\frac{\text{Mass waste}}{\text{Mass product}}$	36.27	45.43

3.2.3. Synthesis of sophorolipid amine oxides

The sophorolipid tertiary amines **213** were also used for the synthesis of a varied set of sophorolipid amine oxides. Amine oxides possess non-ionic or cationic surfactant properties depending on the pH of the solution. Also here, oxidation of the derivatives can have a great influence on the solubility and biological activity of the derivatives. For example, the introduction of the polar *N*-oxide group increases the solubility of the compounds in water. Moreover, they are known to possess good foaming properties and to increase the skin compatibility of detergent products.[170]

At first, the oxidation reaction was attempted with hydrogen peroxide which was considered as a green oxidizing agent since only water would be formed as byproduct. The reaction was performed in methanol at room temperature with 2 equivalents of hydrogen peroxide (Scheme 71).[117] The excess of hydrogen peroxide was destroyed upon addition of 5 mg of platinum black. However, although

some oxidation occurred, no complete conversion towards sophorolipid amine oxides **229** was obtained after 48 hours. When sodium bisulfite was used for the destruction of excess hydrogen peroxide, only starting product could be detected. Probably, sodium bisulfite reduces the formed sophorolipid amine oxide back to the sophorolipid amine.



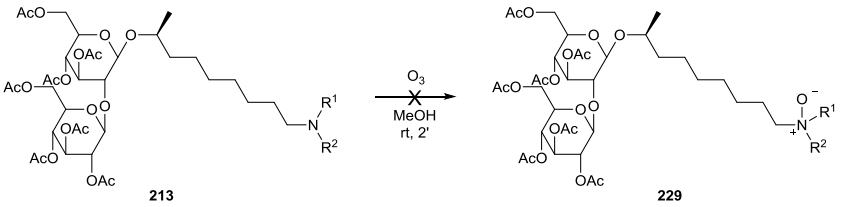
Scheme 71. Synthesis of sophorolipid amine oxides 229 by oxidation with hydrogen peroxide

When the reaction was performed in methanol with 5 equivalents of hydrogen peroxide and 2 equivalents of sodium hydroxide at 50 °C, complete conversion towards the deprotected sophorolipid amine oxides was obtained.[171] However, the deprotected sophorolipid amine oxides could not be purified easily, neither for reaction with sodium hydroxide or sodium methoxide. Several attempts were performed to oxidize sophorolipid amines **213** with hydrogen peroxide without deprotection of the carbohydrate head. Performing the reaction in methanol with 5 equivalents of hydrogen peroxide and 5 wt % dimethyl carbonate at 50 °C was not successful after 3 hours. Different reaction conditions were evaluated for the oxidation of sophorolipid amines **213** in a pressure vial at elevated temperatures (Table 15). All reactions were performed with 5 equivalents of hydrogen peroxide. In all cases, synthesis of the desired sophorolipid amine oxides was detected, but partial or complete deprotection of the carbohydrate head took place during the reaction.

Table 15. Reaction conditions for sophorolipid amine oxidation with 5 equivalents of hydrogen peroxide in a pressure vial

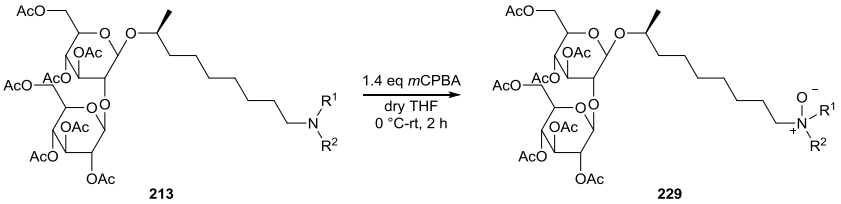
Entry	Solvent	Temperature (°C)	Time (h)	Work-up	Deprotection
1	MeOH	90	3	Fe_2O_3	Partial
2	MeOH	90	2	Fe_2O_3	Partial
3	MeOH	50	1	Fe_2O_3	Partial
4	ACN	50	1	Fe_2O_3	Partial
5	MeOH	60	1	Pt black	Partial
6	MeOH (+ 0.15 eq NaOMe)	60	1	Fe_2O_3	Complete

Oxidation of sophorolipid amines **213** was also evaluated *via* an ozonolysis reaction. In the work of the research group of Patrick Dussault, it is described that the ozonolysis of tertiary amines results in the formation of amine oxides, a procedure which was exploited by the researchers to prevent the formation of stable ozonide intermediates (*vide supra*).[114] The reaction was performed in methanol which was sparged with ozone for 2 minutes (Scheme 72). Although the synthesis of peracetylated sophorolipid amine oxides **229** was detected, complicated reaction mixtures were mostly obtained.



Scheme 72. Synthesis of sophorolipid amine oxides 229 by oxidation with ozone

Finally, 3-chloroperoxybenzoic acid (*m*CPBA) was evaluated for the synthesis of sophorolipid amine oxides **229**. The reaction was performed in dry THF with 1.4 equivalents of *m*CPBA, first 30 minutes at 0 °C and then 90 minutes at room temperature.[172] With *m*CPBA as oxidizing agent, complete conversion to the desired peracetylated sophorolipid amine oxides **229** was easily accomplished (Scheme 73). However, different conditions had to be evaluated to remove the benzoic acid byproduct from the reaction mixture. At first, the purification of sophorolipid amine oxides **229** was attempted *via* filtration over a basic alumina column when dissolved in ethyl acetate.[172] Elution with methanol should furnish the purified amine oxides. However, benzoic acid was still present after the purification step. Secondly, purification was attempted *via* addition of potassium carbonate to the reaction and filtration of the solids. Also here, benzoic acid was still present after the purification step. Finally, the sophorolipid amine oxides **229** could be purified via a washing step with sodium bicarbonate when dissolved in ethyl acetate. Sophorolipid amine oxides **229** were obtained in high purity and did not require further purification. A set of seven different peracetylated sophorolipid amine oxides **229** was synthesized (Table 16, Figure 24).



Scheme 73. Synthesis of sophorolipid amine oxides 229 by oxidation with *m*CPBA

Table 16. Yield for the synthesis of sophorolipid amine oxides 229

Sophorolipid amine	Yield (%)	
213a	**229a**	79
213c	**229b**	87
213d	**229c**	93
213e	**229d**	95
213f	**229e**	94
213g	**229f**	63
213h	**229g**	92

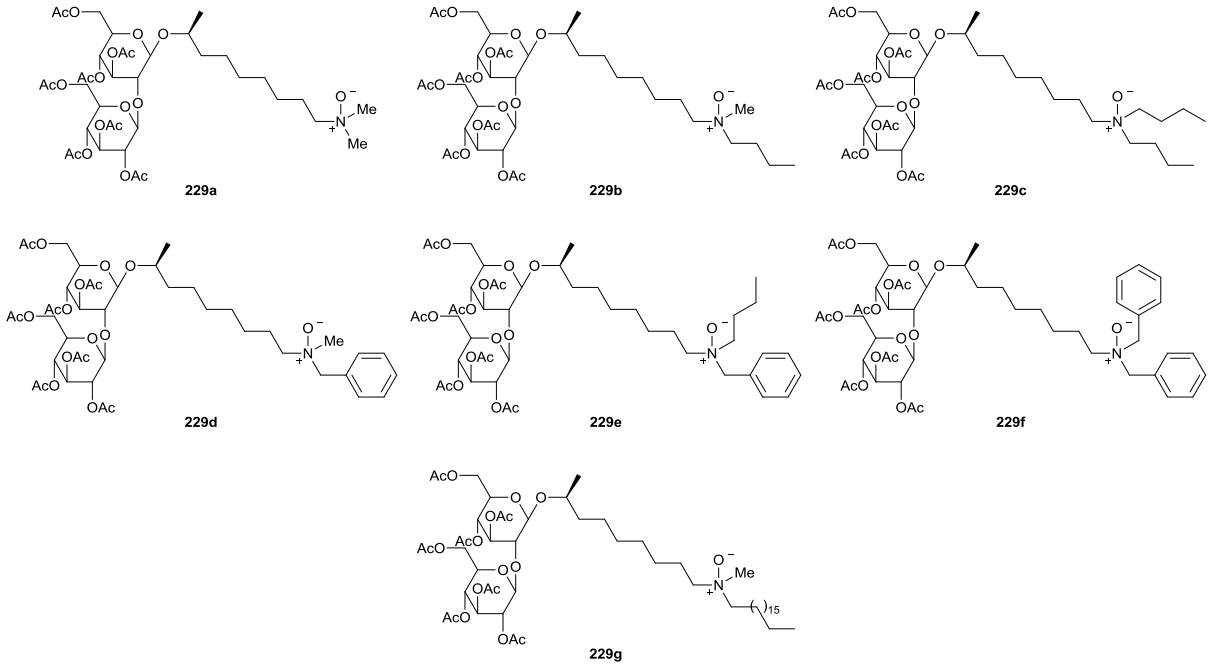
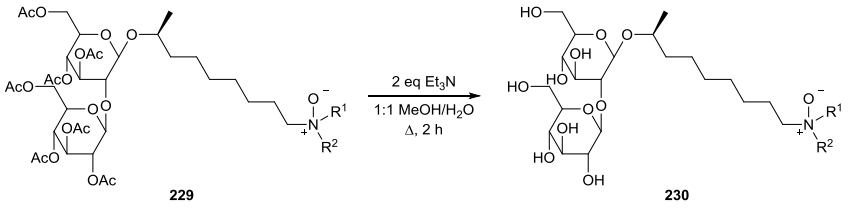


Figure 24. Library of peracetylated sophorolipid amine oxides 229

This set of seven peracetylated sophorolipid amine oxides was deprotected to obtain water soluble amine oxide derivatives. This deprotection was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions (Scheme 74).[135] Evaporation of the reagent, solvent and methyl acetate byproduct yielded pure sophorolipid amine oxides **230**. This also resulted in the synthesis of a set of seven different sophorolipid amine oxides (Table 17, Figure 25).



Scheme 74. Deprotection towards sophorolipid amine oxides 230

Table 17. Yield for the synthesis of sophorolipid amine oxides 230

Sophorolipid amine oxide	Yield (%)	
229a	**230a**	quant.
229b	**230b**	95
229c	**230c**	84
229d	**230d**	quant.
229e	**230e**	91
229f	**230f**	quant.
229g	**230g**	81

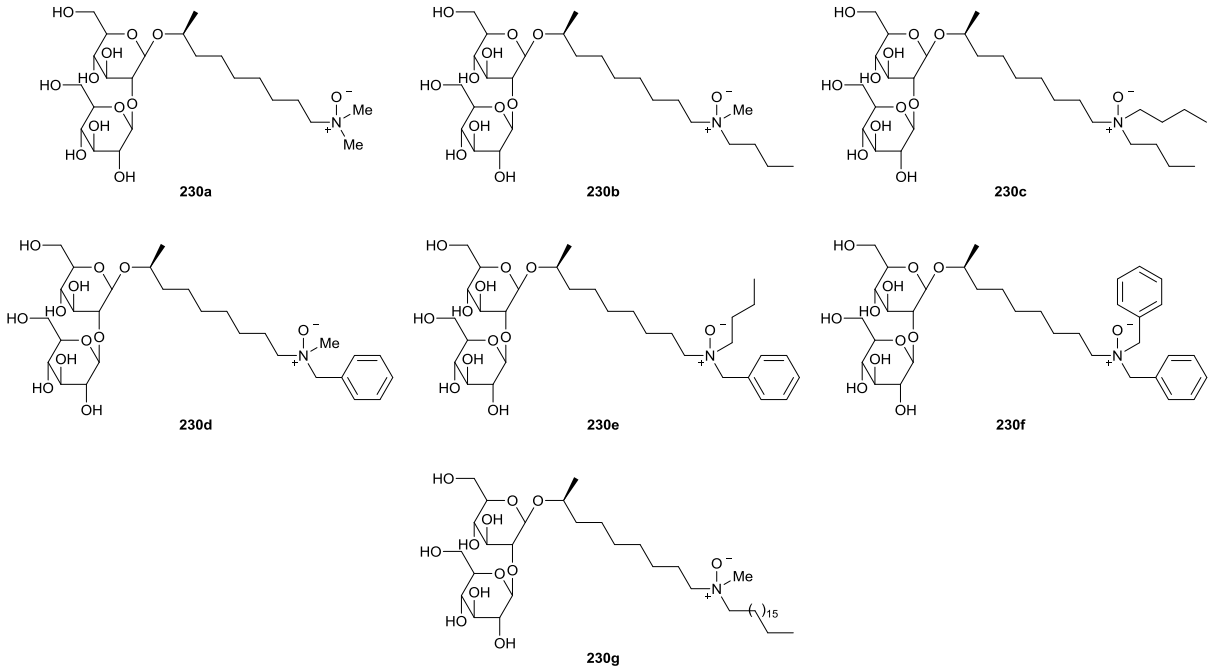


Figure 25. Library of deprotected sophorolipid amine oxides 230

The antimicrobial activity of both peracetylated and deprotected sophorolipid amine oxides **229** and **230** was evaluated. The evaluation of the antimicrobial activities was performed by the Laboratory of Pharmaceutical Microbiology (Ghent University). The Gram-negative bacteria *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095 and *Pseudomonas aeuruginosa* PAO1, and the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 were chosen as test organisms. The bioassay was carried out in 96-well plates in a concentration series ranging from 1000 to 0.48 µg/mL of test compound and approximately 5 x 10^4 bacteria in a final volume of 200 µL. Only sophorolipid amine oxide **230f** showed weak activity against *Pseudomonas aeruginosa* PAO1 with a $MIC_{1/2}$ value of 1000 µg/mL. These results demonstrate that the quaternary ammonium group of the quaternary ammonium sophorolipids **220** and **221** was the key factor to induce the antimicrobial activities, since no antimicrobial activity was demonstrated for these very similar sophorolipid amine oxides.

3.2.4. Synthesis of bolaamphiphilic sophorolipids

Bolaamphiphilic derivatives are amphiphilic compounds which contain two hydrophilic parts linked by a hydrophobic linker. Currently, there is a lot of interest in synthetic bolaamphiphiles because of the unusual architectures created by such molecules.[173] They form monolayer membranes which can organize into micelles, vesicles, nanotubuli, *etc*. These configurations often arise spontaneously, they are self-organizing structures. The best known natural examples of bolaamphiphiles are the tetraether lipid membranes of the archaeabacteria which are able to grow under very extreme temperatures and at very high salt concentrations. These features are supposed to be linked to the

stability of the membranes due to the presence of the bolaamphiphiles. As a result, they can for example be used in drug delivery applications to form or stabilize vesicles. Most liposomes currently used for this purpose face stability problems.[174] Moreover, bolaamphiphiles can be used as membrane-spanning linkers in biosensors to detect proteins, antibodies, viruses, *etc*.[175] They can also serve as channels for ion transport.[173] Fluorescent bolaamphiphiles can be used as transmembrane probes for lipid imaging to visualize for example ether lipids in the brain.[176]

A first set of bolaamphiphilic sophorolipid derivatives was synthesized *via* reductive amination of sophorolipid aldehyde **201** with several diamines. A set of secondary diamines **231** with an ethylene-, hexamethylene- or *o*-phenylenelinker and methyl, butyl or octadecyl groups was selected for the synthesis of the desired bolaamphiphilic sophorolipids (Figure 26). Since only *N*,*N*'-dimethylethylenediamine **231a** and *N*,*N*'-dimethylhexamethylenediamine **231b** are commercially available, other secondary diamines had to be synthesized.

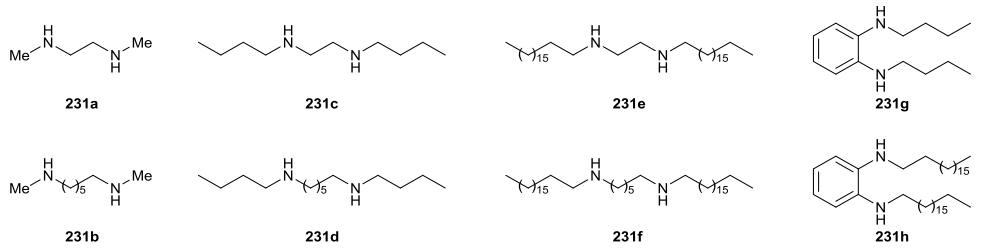
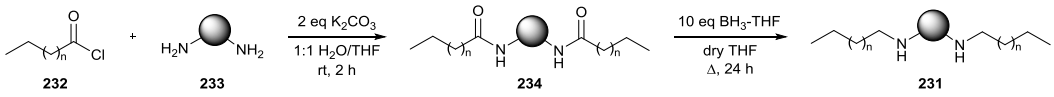


Figure 26. N,N'-dialkyldiamines 231 as substrates for the reductive amination towards bolaamphiphilic sophorolipids

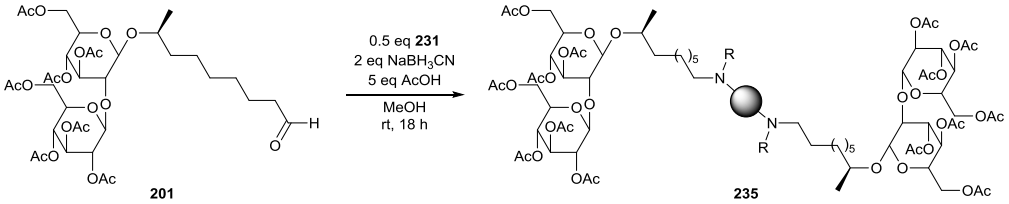
The intermediate diamides **234** were synthesized *via* a Schotten-Bauman reaction with acid chlorides **232** and diamines **233** (Scheme 75). Subsequently, these intermediate diamides were reduced with a borane tetrahydrofuran complex towards the desired *N*,*N*'-dialkyldiamines **231**. With this procedure, a total set of eight different *N*,*N*'-dialkyldiamines **231** was available, including the commercially available *N*,*N*'-dimethylethylenediamine **231a** and *N*,*N*'-dimethylhexamethylenediamine **231b**.



Scheme 75. Synthesis of *N*,*N*'-dialkyldiamines 231

The synthesis of the desired bolaamphiphilic sophorolipids **235** was performed with 0.5 equivalents of *N*,*N*'-dialkyldiamines **231** according to the reaction conditions described for the synthesis of the tertiary sophorolipid amines **213** (Scheme 76). The reductive amination of sophorolipid aldehyde **201** with *N*,*N*'-dialkyldiamines **231a-d** resulted in the synthesis of the desired bolaamphiphilic sophorolipids **235a-d** (Table 18). The compounds were purified *via* automated column chromatography with a hexane/ethyl acetate/triethylamine mixture as eluent. However, reductive amination with *N*,*N*'-dialkyldiamines **231e-h** was not successful. In the case of

N,*N*'-dioctadecyldiamines **231e**, **231f** and **231h**, a poor solubility of the diamines in the reaction solvent prevented the synthesis of the desired bolaamphiphilic sophorolipids. When tetrahydrofuran was evaluated as solvent instead of methanol, no reductive amination occurred either. In the case of *N*,*N*'-dibutyl-*o*-phenylenediamine **231g**, the reductive amination did occur but no complete conversion could be obtained due to the steric hindrance caused by the close proximity of the two butyl groups.

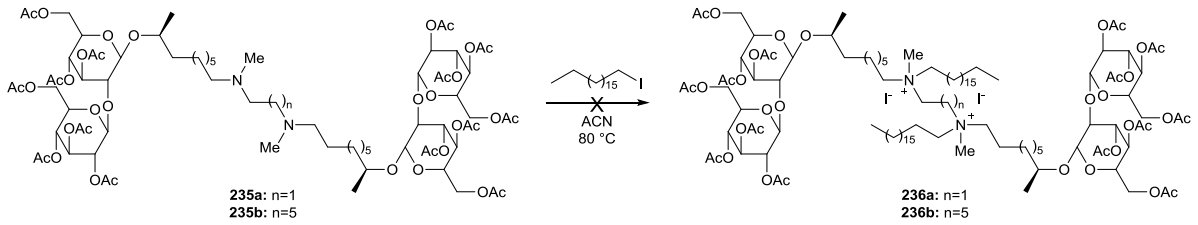


Scheme 76. Synthesis of bolaamphiphilic sophorolipids 235 *via* reductive amination with *N*,*N*'dialkyldiamines 231

Table 18. Yield for the synthesis of *N*,*N*-dialkyl bolaamphiphilic sophorolipids 235

Bolaamphiphilic sophorolipid	R-group	Linker	Yield (%)
235a	Me	ethylene	31
235b	Me	hexamethylene	31
235c	Bu	ethylene	41
235d	Bu	hexamethylene	27
235e	$C_{18}H_{37}$	ethylene	/
235f	$C_{18}H_{37}$	hexamethylene	/
235g	Bu	*o*-phenylene	/
235h	$C_{18}H_{37}$	*o*-phenylene	/

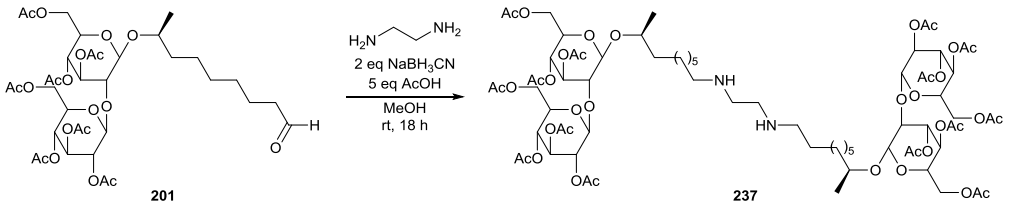
Alternative procedures were evaluated for the synthesis of bolaamphiphilic sophorolipids with octadecyl groups on the nitrogen atom. At first, the introduction of the octadecyl group was attempted *via* quaternization of the *N*,*N*'-dimethyl bolaamphiphilic sophorolipids **235a** and **235b** with octadecyl iodide. However, no quaternization occurred with 2 equivalents of octadecyl iodide in acetonitrile or toluene as solvent after 72 hours (Scheme 77).



Scheme 77. Quaternization of *N*,*N*'-dimethyl bolaamphiphilic sophorolipids 235a and 235b with octadecyl iodide

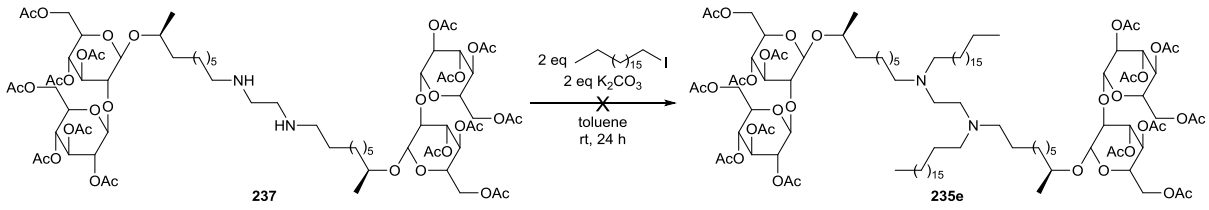
Therefore, an alternative procedure was designed which comprised the formation of secondary bolaamphiphilic sophorolipid amines as intermediates. The synthesis of a set of secondary

bolaamphiphilic sophorolipid amines was attempted *via* reductive amination with primary diamines. At first, the synthesis was evaluated with 0.5 equivalents of primary diamines according to the reaction conditions described for the synthesis of the tertiary sophorolipid amines **213**. With this procedure, a broad reaction mixture was obtained. In view of the results obtained for the synthesis of the secondary sophorolipid amines **214**, similar overalkylations will cause the formation of this broad reaction mixture. Therefore, the mixture of sophorolipid aldehyde **201** and diamine was also stirred for 1 hour at room temperature prior to the addition of sodium cyanoborohydride and acetic acid (Scheme 78). This procedure proved to be successful with ethylenediamine but not with *o*-phenylenediamine.



Scheme 78. Synthesis of bolaamphiphilic sophorolipids 237 *via* reductive amination with primary diamines

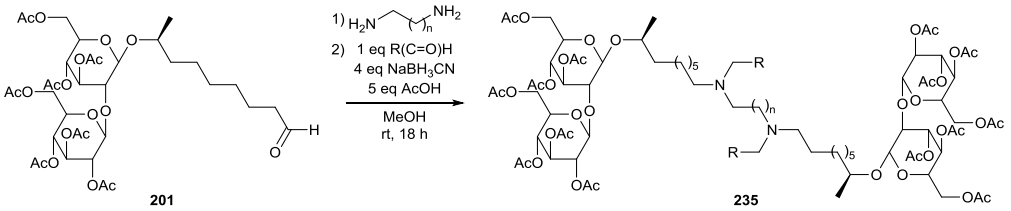
Subsequently, an attempt was made to alkylate the ethylenediamine bolaamphiphilic sophorolipid **237** with octadecyl iodide. No purification was performed prior to the alkylation. Therefore, no yield was determined for this intermediate. The reaction was performed with 2 equivalents of octadecyl iodide in toluene for 24 hours at room temperature (Scheme 79). However, also this procedure was not successful for the synthesis of *N*,*N*'-dioctadecyl bolaamphiphilic sophorolipid **235e**. Only starting compound could be detected *via* NMR analysis.



Scheme 79. Alkylation of ethylenediamine bolaamphiphilic sophorolipid 237 with octadecyl iodide

Finally, two reductive aminations in sequence were evaluated for the synthesis of *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **235** (Scheme 80). First, the mixture of sophorolipid aldehyde **201** and diamine was stirred for 1 hour at room temperature as previously described. Subsequently, 1 equivalent of a second aldehyde is added, together with 4 equivalents of sodium cyanoborohydride and 5 equivalents of acetic acid. The reaction mixture was stirred overnight at room temperature and the reaction work-up was performed similarly as for the other reductive aminations. This dual reductive amination procedure was successful for the synthesis of *N*,*N*'-dibutyl bolaamphiphilic sophorolipid **235c**, although the reaction product was less pure than the one obtained after reductive

amination with *N*,*N*'-dibutyl ethylene diamine **234c**. However, when this dual reductive amination was applied for the synthesis of *N*,*N*'-dioctadecyl bolaamphiphilic sophorolipids **235e** and **235f**, the formation of the desired compounds could not be observed by NMR. Therefore, a set of only four different *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **235** could successfully be synthesized (Figure 27).



Scheme 80. Dual reductive amination towards *N*,*N*'dialkyl bolaamphiphilic sophorolipids 235

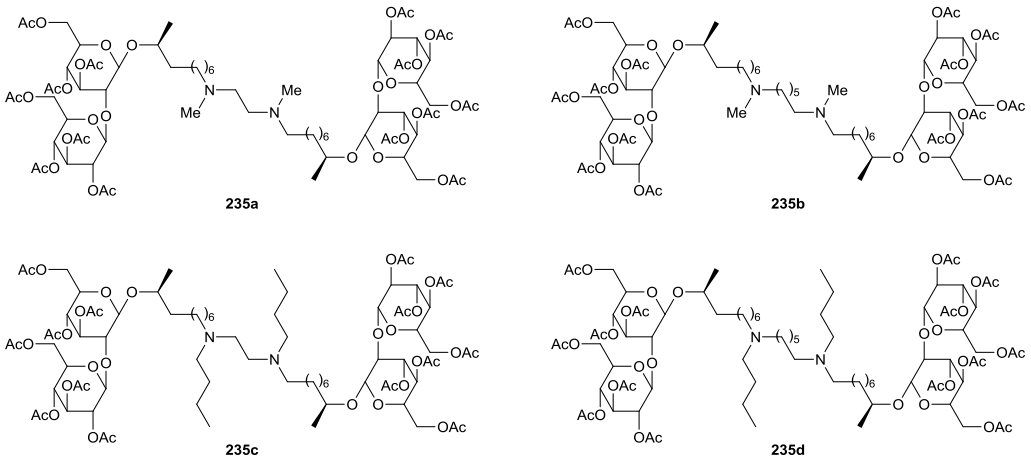
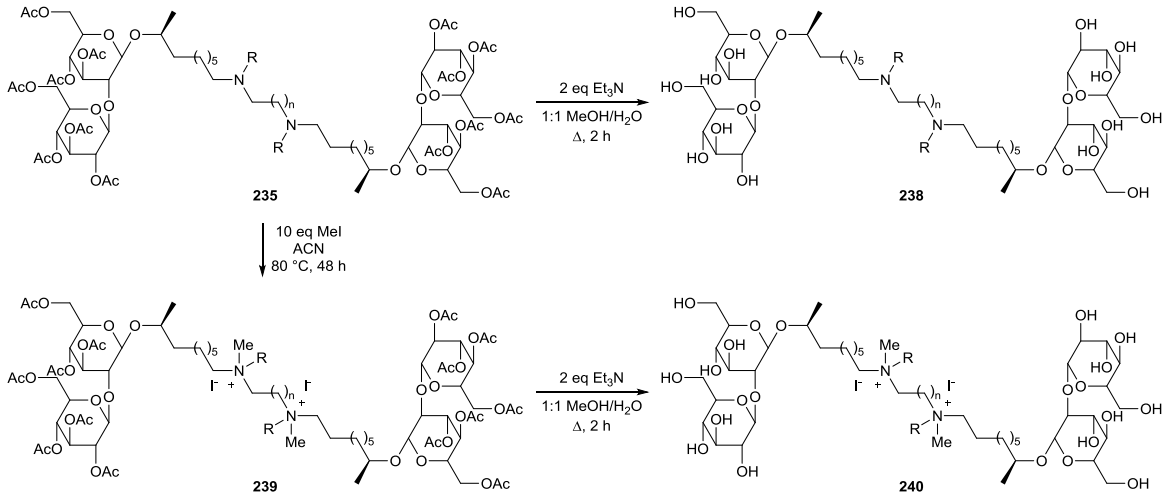


Figure 27. Library of N,N'-dialkyl bolaamphiphilic sophorolipids 235

This set of four *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **235** was transformed into the deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **238**, peracetylated dicationic bolaamphiphilic sophorolipids **239** and deprotected dicationic bolaamphiphilic sophorolipids **240** (Scheme 81). The deprotection was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions as was already described for the amine oxides. Evaporation of the reagent, solvent and the methyl acetate byproduct yielded pure deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **238**, resulting in the synthesis of a set of four different derivatives (Table 19, Figure 28).



Scheme 81. Modification of *N*,*N*'-dialkyl bolaamphiphilic sophorolipids 235 *via* quaternization and deprotection

Table 19. Yield deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipids 238

Bolaamphiphilic sophorolipid	Yield (%)	
235a	**238a**	94
235b	**238b**	95
235c	**238c**	91
235d	**238d**	89

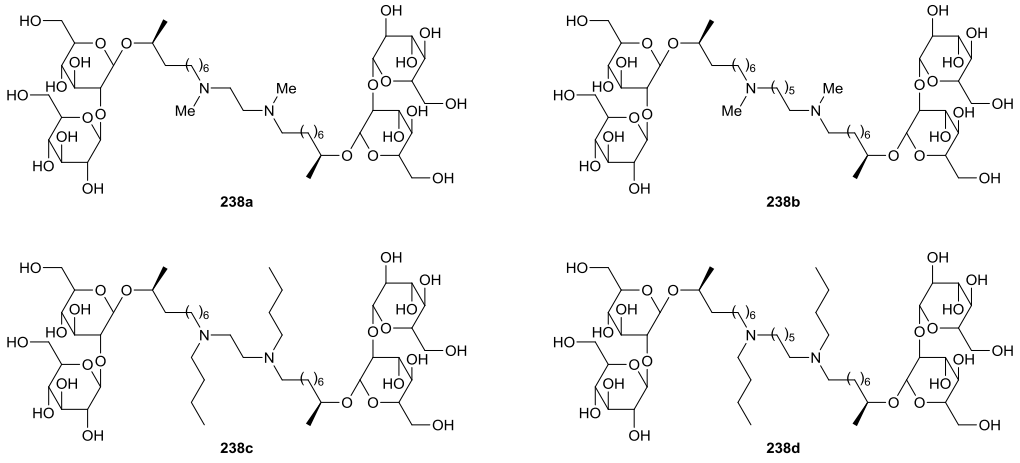


Figure 28. Library of deprotected N,N'-dialkyl bolaamphiphilic sophorolipids 238

The quaternization was performed in a pressure vial with 10 equivalents of methyl iodide. After 48 hours, complete conversion towards the dicationic bolaamphiphilic sophorolipids **239** was obtained and no further purification of the derivatives was required. This resulted in the synthesis of a set of four different peracetylated dicationic bolaamphiphilic sophorolipids (Table 20, Figure 29). In a previous research project at our research group, the double quaternization of a bis(7-azabicyclo[2.2.1.]heptane) derivative with an ethylene linker to a dicationic compound proved to be unsuccessful, even upon reaction with 20 equivalents of methyl iodide at room temperature.[177] Therefore, it could be anticipated that the large charge repulsion on the small ethylene linker could

also inhibit the formation of the dicationic bolaamphiphilic sophorolipids **239a** and **239c**. However, NMR-analysis clearly confirmed the successful synthesis of both compounds.

Table 20. Yield peracetylated dicationic bolaamphiphilic sophorolipids 239

Bolaamphiphilic sophorolipid	Yield (%)	
235a	**239a**	quant.
235b	**239b**	quant.
235c	**239c**	93
235d	**239d**	94

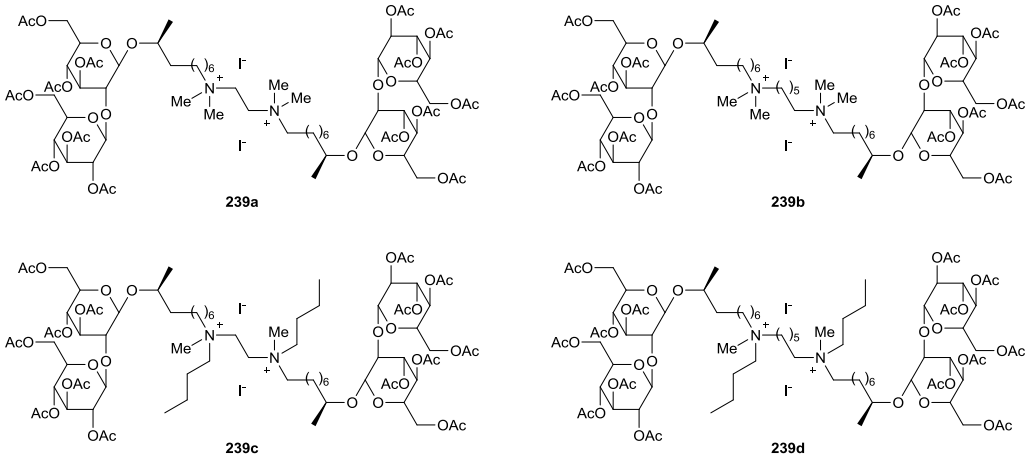


Figure 29. Library of peracetylated dicationic bolaamphiphilic sophorolipids 239

The deprotection of the quaternary ammonium bolaamphiphilic sophorolipids **239** was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions as was already described before. Evaporation of the reagent, solvent and methyl acetate byproduct yielded pure deprotected dicationic bolaamphiphilic sophorolipids **240**. This resulted in the synthesis of a set of four different deprotected dicationic bolaamphiphilic sophorolipids (Table 21, Figure 30).

Table 21. Yield deprotected dicationic bolaamphiphilic sophorolipids 240

Quaternary ammonium bolaamphiphilic sophorolipid	Yield (%)	
239a	**240a**	96
239b	**240b**	96
239c	**240c**	79
239d	**240d**	94

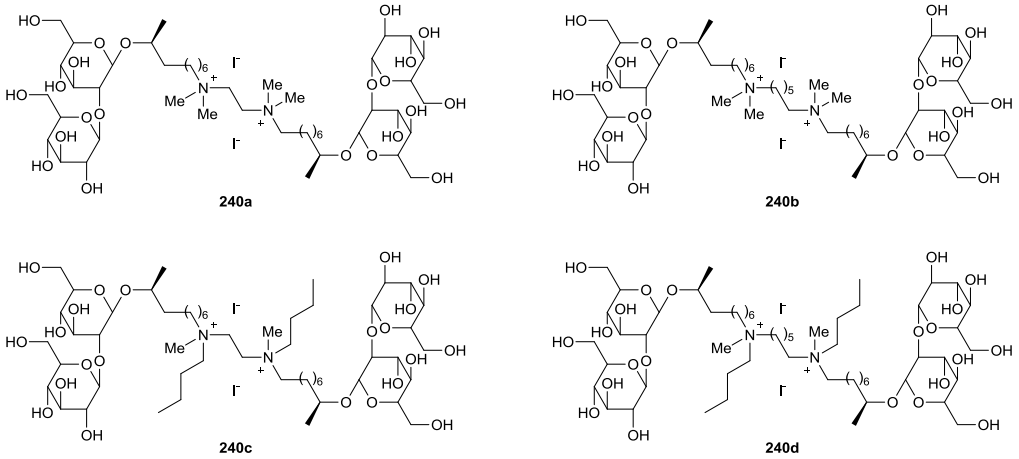
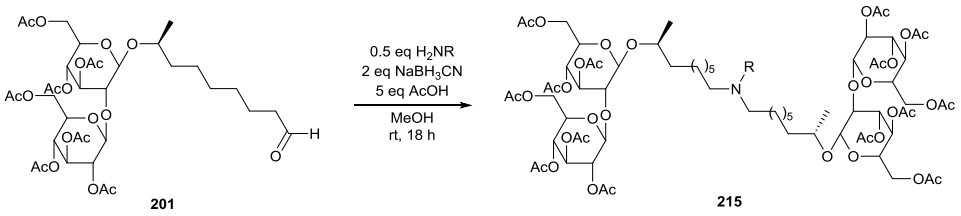


Figure 30. Library of deprotected dicationic bolaamphiphilic sophorolipids 240

Another class of bolaamphiphilic sophorolipids was synthesized *via* the reductive amination of sophorolipid aldehyde **201** with primary amines (*vide supra*). The selective formation of *N*-alkyl bolaamphiphilic sophorolipid amines **215** was accomplished by using only 0.5 equivalents of primary amine and applying the same reaction conditions as described for the reductive amination with the secondary amines (Scheme 82). A set of four different peracetylated *N*-alkyl bolaamphiphilic sophorolipid amines **215** was synthesized. Purification was first attempted *via* automated column chromatography with different gradients of a hexane/ethyl acetate/triethylamine mixture as eluent. However, this purification was only successful for *N*-methyl bolaamphiphilic sophorolipid **215a**. For the other three derivatives, the purification was performed *via* preparative TLC with ethyl acetate as eluent, resulting in pure peracetylated *N*-alkyl bolaamphiphilic sophorolipid amines **215b-d** (Table 22, Figure 31).



Scheme 82. Synthesis of bolaamphiphilic sophorolipids 215 *via* reductive amination with primary amines

Table 22. Yield for the synthesis of bolaamphiphilic sophorolipids 215

Bolaamphiphilic sophorolipid	R-group	Yield (%)
215a	Me	55
215b	Bu	15
215c	Bn	39
215d	$C_{18}H_{37}$	18

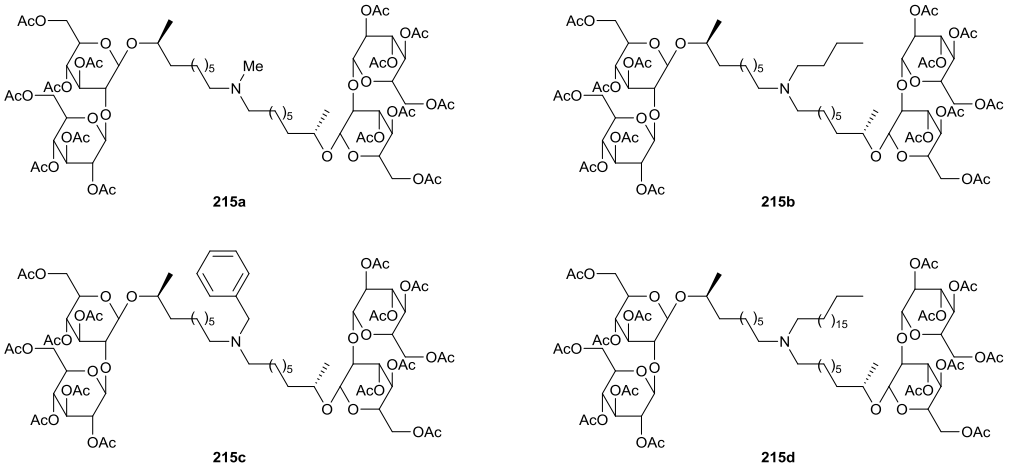
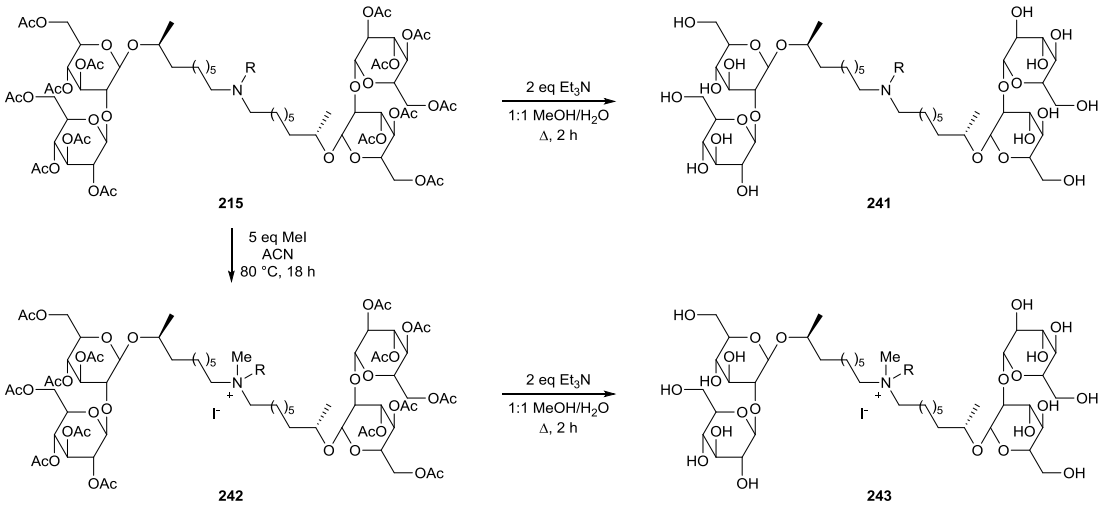


Figure 31. Library of *N*-alkyl bolaamphiphilic sophorolipids 215

This set of four peracetylated *N*-alkyl bolaamphiphilic sophorolipids **215** was transformed into the deprotected *N*-alkyl bolaamphiphilic sophorolipids **241**, peracetylated monocationic bolaamphiphilic sophorolipids **242** and deprotected monocationic bolaamphiphilic sophorolipids **243** (Scheme 83). The deprotection was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions. Evaporation of the reagent, solvent and the methyl acetate byproduct yielded pure deprotected *N*-alkyl bolaamphiphilic sophorolipids **241**, resulting in the synthesis of a set of four different deprotected *N*-alkyl bolaamphiphilic derivatives (Table 23, Figure 32).



Scheme 83. Modification of *N*-alkyl bolaamphiphilic sophorolipids 215 *via* quaternization and deprotection

Table 23. Yield deprotected *N*-alkyl bolaamphiphilic sophorolipids 241

Bolaamphiphilic sophorolipid	Yield (%)	
215a	**241a**	quant.
215b	**241b**	92
215c	**241c**	80
215d	**214d**	99

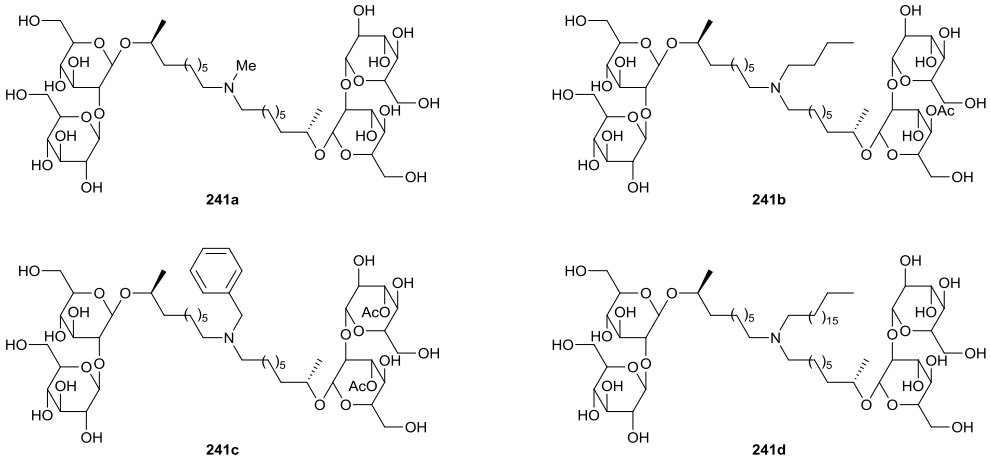


Figure 32. Library of deprotected *N*-alkyl bolaamphiphilic sophorolipids 241

The quaternization was performed in a pressure vial with 5 equivalents of methyl iodide. After 18 hours, complete conversion towards the quaternary ammonium bolaamphiphilic sophorolipids **242** was obtained and no further purification of the derivatives was required. This resulted in the synthesis of a set of four different peracetylated monocationic bolaamphiphilic sophorolipids (Table 24, Figure 33).

Table 24. Yield peracetylated monocationic bolaamphiphilic sophorolipids 242

Bolaamphiphilic sophorolipid	Yield (%)	
215a	**242a**	94
215b	**242b**	95
215c	**242c**	96
215d	**242d**	97

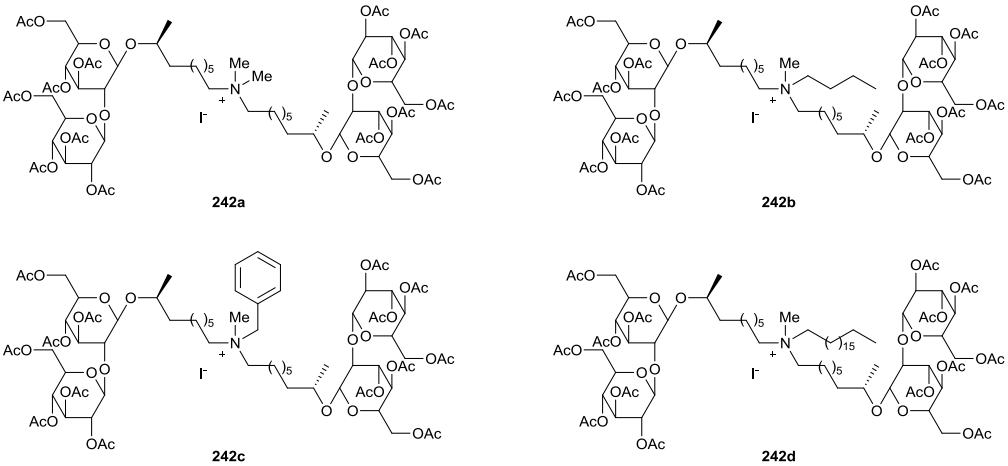


Figure 33. Library of peracetylated quaternary ammonium bolaamphiphilic sophorolipids 242

The deprotection of the peracetylated monocationic bolaamphiphilic sophorolipids **242** was performed with 2 equivalents of triethylamine in a mixture of methanol and water under reflux conditions as was already described before. Evaporation of the reagent, solvent and the methyl acetate byproduct yielded pure deprotected monocationic bolaamphiphilic sophorolipids **243**, resulting in a set of four different deprotected monocationic bolaamphiphilic sophorolipids (Table 25, Figure 34).

Table 25. Yield deprotected monocationic bolaamphiphilic sophorolipids 243

Bolaamphiphilic sophorolipid	Yield (%)	
242a	**243a**	80
242b	**243b**	96
242c	**243c**	96
242d	**243d**	95

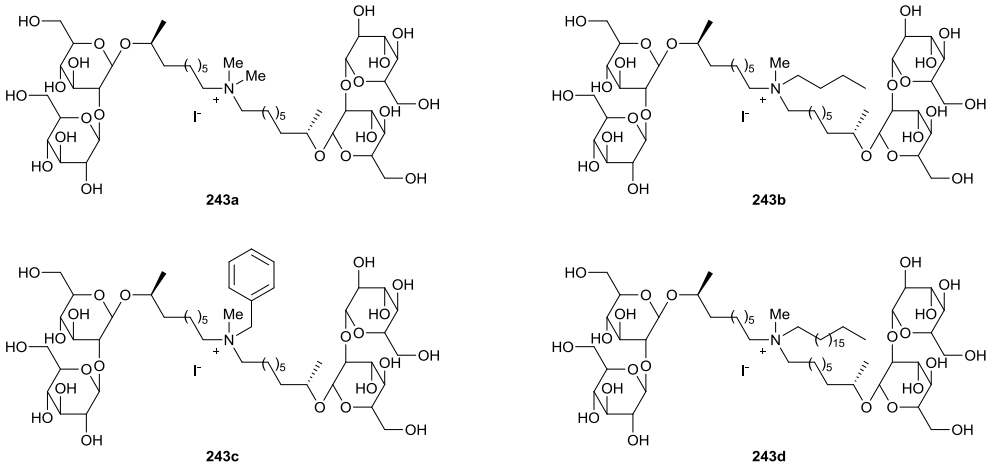


Figure 34. Library of deprotected monocationic bolaamphiphilic sophorolipids 243

The antimicrobial activity of the peracetylated and deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **235** and **238**, peracetylated and deprotected dicationic bolaamphiphilic sophorolipids **239** and **240**, peracetylated and deprotected *N*-alkyl bolaamphiphilic sophorolipids **215** and **241**, and peracetylated and deprotected monocationic bolaamphiphilic sophorolipids **242** and **243** was evaluated. The evaluation of the antimicrobial activities was performed by the Laboratory of Pharmaceutical Microbiology (Prof. T. Coenye, Ghent University). The Gram-negative bacteria *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095 and *Pseudomonas aeruginosa* PAO1, and the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 were chosen as test organisms. The bioassay was carried out in 96-well plates in a concentration series ranging from 2500 to 1.22 µg/ml of test compound and approximately 5 x 10^4 bacteria in a final volume of 200 µL. None of the evaluated compounds showed activity against the three Gram-negative strains. A set of eleven bolaamphiphilic sophorolipids showed activity against one or both of the Gram-positive strains (Table 26).

Table 26. Minimum inhibitory concentrations (µg/ml) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

	235b	**239a**	**239b**	**239c**	**239d**	**241b**	**242a**	**242b**	**242c**	**243c**	**243d**
***S. aureus* ATCC 6538**	625	312	156	39	78	625	156	78	78	156	156
***S. aureus* Mu50**	2500	1250	625	312	78	>2500	312	78	78	>2500	312

For a better comparison of the evaluated derivatives with each other and with the previous set of quaternary ammonium sophorolipids, the MIC values were converted based on their molecular weight (Table 27). Within this set of bolaamphiphilic sophorolipid derivatives, the peracetylated dicationic and monocationic congeners **239** and **242** proved to be the most active. The highest activity was obtained for peracetylated *N*,*N*'-dibutyl,*N*,*N*'-dimethyl ethylene bolaamphiphilic sophorolipid diaminium diodide **239c**. However, this MIC value is still a tenfold higher than the one obtained for the deprotected quaternary ammonium sophorolipids **221h** and **221i**.

Table 27. Minimum inhibitory concentrations (µM) against *S. aureus* ATCC 6538 and *S. aureus* Mu50

	235b	**239a**	**239b**	**239c**	**239d**	**241b**	**242a**	**242b**	**242c**	**243c**	**243d**
***S. aureus* ATCC 6538**	375	165	80	20	38	392	92	45	44	132	116
***S. aureus* Mu50**	1501	660	321	158	38	>1569	184	45	44	>2114	232

3.2.5. Conclusions

Four different classes of sophorolipid derivatives were successfully synthesized: intermediate sophorolipid amines, quaternary ammonium sophorolipids, sophorolipid amine oxides and bolaamphiphilic sophorolipids.

The purity of the sophorolipid amines after reductive amination proved to be highly dependent on the quality of the sophorolipid aldehyde. No extra purification step was needed after reductive amination with highly pure sophorolipid aldehyde which was derived from ozonolysis reactions with dichloromethane as solvent. However, further purification proved to be necessary in case methanol was used as solvent for the ozonolysis reaction due to the slightly decreased purity of this ozonolysis product. Therefore, a balance should be found between the green character of the ozonolysis solvent and its influence on the purity and concomitant purification steps of the sophorolipid amine derivatives after reductive amination. As stated before, the use of continuous flow microreactor technology could offer a solution to increase the purity of the sophorolipid aldehyde synthesized *via* ozonolysis in methanol.

All derivatives have been evaluated for their antimicrobial activity against a set of Gram-positive and Gram-negative strains. None of the derivatives displayed significant activity against any of the Gram-negative strains. The quaternary ammonium sophorolipids, monocationic bolaamphiphilic sophorolipids and dicationic sophorolipids proved to be the most active derivatives. The best results were obtained for the deprotected quaternary ammonium sophorolipids which possess an octadecyl chain on the nitrogen atom (Figure 35). These derivatives were even more active than the antibiotic gentamicin sulfate against the four Gram-positive strains *S. aureus*, *E. faecium*, *B. subtilis* and *S.*

pneumoniae in *in vitro* evaluations. Moreover, evaluation of the deglycosylated derivatives of these two quaternary ammonium sophorolipids demonstrated that the presence of the carbohydrate head has a positive effect on the antimicrobial activity.

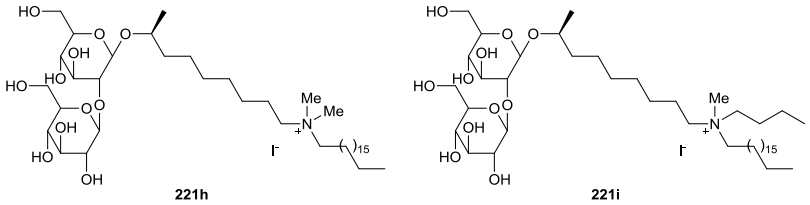


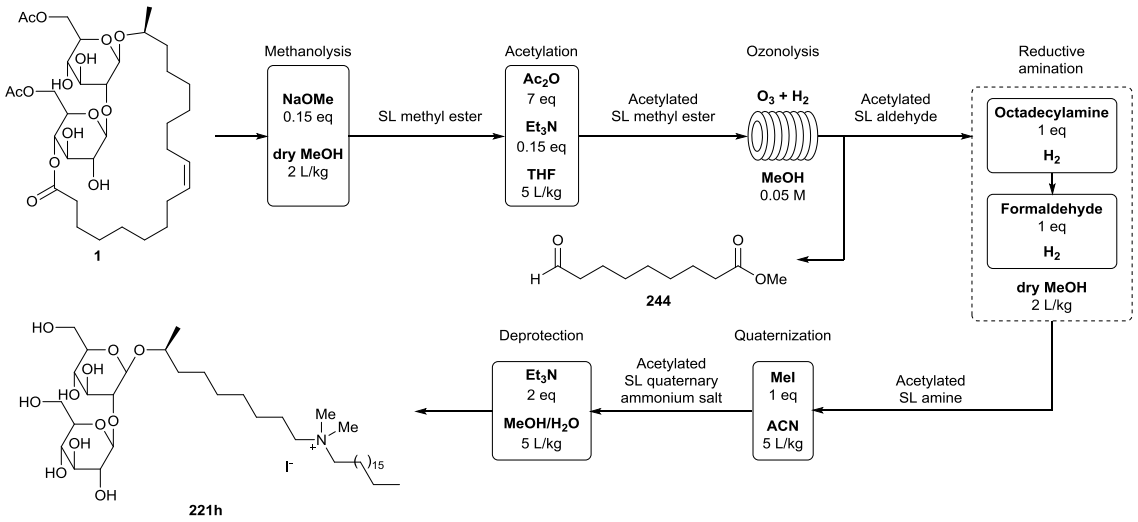
Figure 35. Quaternary ammonium sophorolipids 221h and 221i

Evaluation of the quaternary ammonium sophorolipids on their suitability as gene delivery vectors indicated that these same two quaternary ammonium sophorolipids with an octadecyl chain were the most efficient derivatives. Moreover, evaluation of their deglycosylated derivatives demonstrated that the presence of the carbohydrate head highly increased the cell viability of the evaluated cell lines and concomitant biocompatibility of these quaternary ammonium sophorolipids.

In view of their biological activities, quaternary ammonium sophorolipids **221h** and **221i** can be regarded as the best candidates for pharmaceutical applications and for further property evaluation. The presence of the carbohydrate head proved to be necessary for a high antimicrobial activity against Gram-positive strains and an efficient transfection with high cell viability. Although sophorolipid amine oxide **230g** displays a very similar structure, no antimicrobial activity was observed for this compound. This indicates that the quaternary ammonium group is also a key factor to induce the antimicrobial activity. The evaluation of some green chemistry metrics for the synthesis of quaternary ammonium sophorolipids **221h** and **221i** indicated that the E-factors for their synthetic pathways lie between the range of E-factors for fine chemicals (5-50) and pharmaceuticals (25-100).

3.3. Perspectives

In view of their antimicrobial properties and transfection efficiencies, the deprotected quaternary ammonium sophorolipids with an octadecyl chain on the nitrogen atom proved to be the best candidates for further optimization and industrial applications. As stated in the introduction, the focus has to be on high added-value applications for the pharmaceutical sector to render the new derivatives economically competitive. Before the production process can be applied on a commercial scale, changes have to be made to some of the reaction steps (Scheme 84).



Scheme 84. Flow chart for the commercial production of deprotected quaternary ammonium sophorolipid 221h

First of all, the ozonolysis reaction proved to be the bottleneck of this synthetic pathway. The choice of the reaction solvent proved to have a big influence on the purity of the sophorolipid aldehyde intermediate and on the necessity of a subsequent purification step of the sophorolipid amines. The use of continuous flow microreactor technology ensures a higher control of the reaction parameters, resulting in a higher selectivity and an increase in the process efficiency and safety. Moreover, it would enable the safe scale-up, eventually to a commercial scale, of this potential dangerous reaction. Reductive work-up of the ozonolysis reaction *via* hydrogenation should be evaluated as alternative for sodium triacetoxyborohydride. Automated chromatography purification after this reaction step should be avoided to reduce operating costs and enable the scale-up of the process. Therefore, distillation of the methyl 9-oxononanoate by-product **244**, which has a boiling point of 125-145 °C at 12 mbar, should be examined. This by-product can be valorized as a precursor for biopolymer products.[178]

Secondly, the reductive amination should be performed in two subsequent steps. In the synthesis pathway towards deprotected quaternary ammonium sophorolipid **221h**, a first step comprises the imination of the sophorolipid aldehyde with octadecylamine, followed by hydrogenation towards the intermediate secondary sophorolipid amine. In the second step, imination with formaldehyde and

subsequent hydrogenation results in the synthesis of the desired tertiary sophorolipid amine. With this reaction sequence, the use of the more expensive *N*-methyloctadecylamine and the more hazardous sodium cyanoborohydride reducing agent is avoided. Finally, triethylamine can be used instead of DMAP for the acetylation reaction to reduce product costs and facilitate product purification.

Based on the optimized reaction pathway, an attempt was made to estimate the production price for the deprotected quaternary ammonium sophorolipid **221h** on a 10 kg scale (Table 28). A first scenario is based on the actual reaction yields, a second scenario is based on a yield of 90% for each reaction step and a third scenario is based on an average production yield of 71%.

Table 28. Estimated product price for the production of 10 kg deprotected quaternary ammonium sophorolipid 221h. For three different scenarios, the raw material and solvent demand is calculated in function of the reaction yield.

	Scenario 1	**Scenario 2**	**Scenario 3**
Methanolysis			
Yield (%)	90	90	90
SL lactone (kg)	33	15	11
NaOMe (kg)	0.39	0.17	0.13
Dry MeOH (L)	66	30	22
Acetylation			
Yield (%)	99	90	99
Ac_2O (kg)	30.83	13.58	10.20
Et_3N (kg)	0.65	0.29	0.22
THF (L)	137	60	45
Ozonolysis			
Yield (%)	67	90	90
MeOH (L)	850	350	280
By-product (kg)	5.33	2.86	2.37
Reductive amination			
Yield (%)	40	90	90
Octadecylamine (kg)	7.71	4.15	3.43
Formaldehyde (kg)	0.86	0.46	0.38
Dry MeOH (kg)	44	24	20
Quaternization			
Yield (%)	99	90	99
MeI (kg)	1.62	1.97	1.62
ACN (L)	60	72	60
Deprotection			
Yield (%)	99	90	99
Et_3N (kg)	2.29	2.52	2.29
MeOH (L)	35	37	35
Overall yield (%)	**23**	**53**	**71**
Estimated raw material cost (€)	347	219	174
Estimated solvent cost (€)	1362	846	679
Estimated total cost (€)	2138-5700	1331-3550	1068-2847
Estimated product price (€/kg)	**214-570**	**133-355**	**107-285**

The raw material and solvent costs for the different scenarios are based on current commercial prices for products on kg scale and are estimated to amount to 30-80% of the total production cost.[179] Even in the most optimal scenario, the production price of deprotected quaternary ammonium sophorolipid will still be higher than 100 €/kg. This clearly indicates that the new sophorolipid derivatives will only be economically competitive in high added-value applications, for example for the pharmaceutical sector, if they offer advantages over the currently available products. However, a reduction in the total production cost could still be accomplished if the recycling of solvents and reagents such as triethylamine would be feasible and if the reactions would be performed on a larger scale.

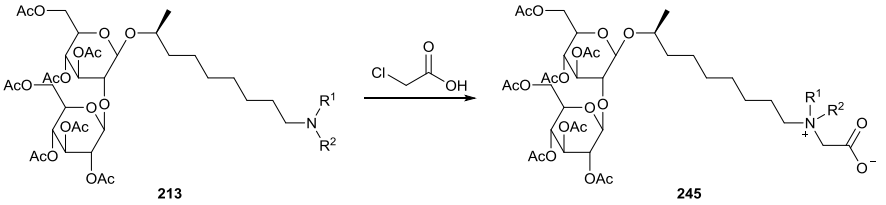
For each scenario, the green chemistry metrics were also calculated (Table 29). Carbon efficiency and atom economy are not dependent on the process conditions and are therefore the same as in Table 14. The assumption was made that stoichiometric amounts of acetic anhydride and methyl iodide can be used, giving a stoichiometric factor (SF) of 1. For each scenario, a distinction was made whether the solvents are considered as waste (A) or can be reused (B). The recycling of solvents offers the possibility to reduce the E-factor more than tenfold.

Table 29. Overview of the green chemistry metrics for the production of quaternary ammonium sophorolipid 221h for the three different scenarios. A distinction is made for each scenario whether the solvents are considered as waste (A) or can be reused (B). RME = reaction mass efficiency, AE = atom economy, SF = stoichiometric factor, ε = reaction yield, c = mass reaction catalyst, s = mass reaction solvent, w = mass reaction waste, m = mass target product.

Parameter	Formula	Scenario 1		Scenario 2		Scenario 3	
		A	B	A	B	A	B
RME (%)	$\varepsilon * (\mathrm{AE}) * \frac{1}{\mathrm{SF}} * \left[\frac{1}{1 + \frac{\varepsilon * (\mathrm{AE}) * (c + s + w)}{(\mathrm{SF}) * m}}\right]$	0.013	0.081	0.024	0.179	0.029	0.235
E-factor (kg/kg)	$\frac{\text{Mass waste}}{\text{Mass product}}$	66.79	4.93	37.17	2.18	31.52	1.69

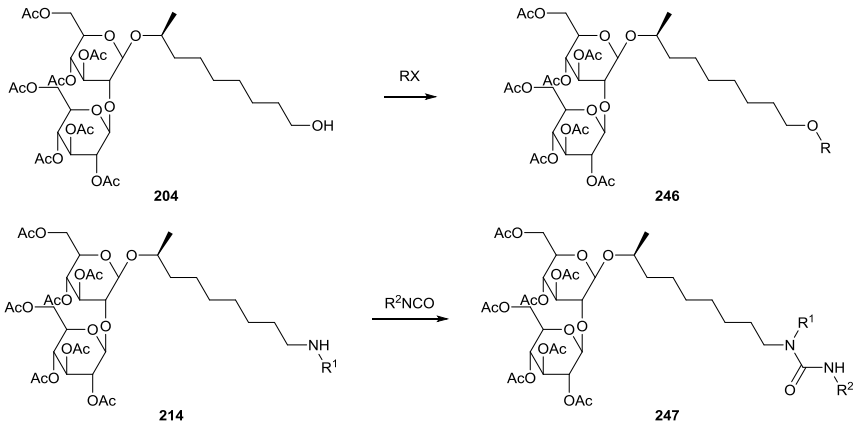
Apart from the commercialization of the currently synthesized sophorolipid derivatives, further research can also focus on the synthesis of new types of sophorolipid derivatives. As stated before, the sophorolipid microbial production pathway limits the fatty acid incorporation to C16 or C18 hydrophobic substrates. In this work, the synthesis of C9 and C12 sophorolipid intermediates was accomplished after incorporation of oleic acid and petroselinic acid, respectively, in the sophorolipid structure. In addition, polyunsaturated fatty acids such as linoleic and linolenic acid can be incorporated in the sophorolipid structure for the synthesis of C6 and C3 sophorolipid intermediates. This will enable a thorough evaluation of the influence of the sophorolipid chain length on their biological and physico-chemical properties.

The set of sophorolipid derivatives can be further extended with the synthesis of other sophorolipid compounds. Sophorolipid betaines **245**, another class of zwitterionic surfactants, can be synthesized from the tertiary sophorolipid amines **213** (Scheme 85). These derivatives will also possess cationic surfactant properties depending on the pH of the solution, which can have a great influence on their solubility and biological activity.

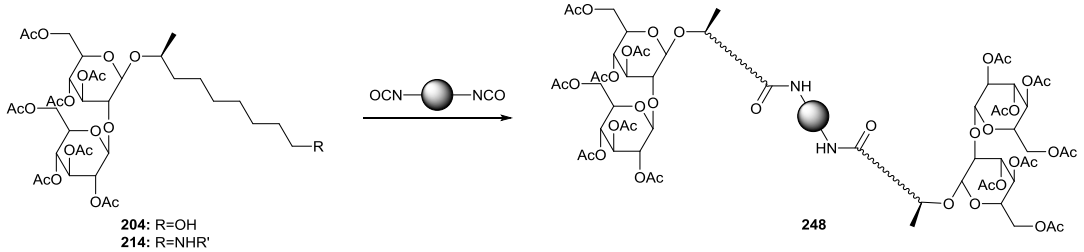


Scheme 85. Proposed synthesis of sophorolipid betaines 245

Sophorolipid ether derivatives **246** and sophorolipid urea derivatives **247**, two types of nonionic surfactants, can be synthesized from sophorolipid alcohol **204** and secondary sophorolipid amines **214**, respectively (Scheme 86). These glyco-ether derivatives and urea derivatives offer perspectives for anti-cancer properties. Sophorolipid alcohol **204** and secondary sophorolipid amines **214** can also be modified with different diisocyanates towards a new class of bolaamphiphilic sophorolipids **248** (Scheme 87). These bolaamphiphilic sophorolipids possess carbamate or urea moieties, which could have an enhanced self-assembly through stabilization of nanostructures *via* hydrogen-bond formation.



Scheme 86. Proposed synthesis of sophorolipid ether derivatives 246 and sophorolipide urea derivatives 247



Scheme 87. Proposed synthesis of a new class of bolaamphiphilic sophorolipids 248

4. Experimental procedures

4.1. Materials and general methods

4.1.1. Materials

The sophorolipid starting compound was obtained from Ecover (Malle, Belgium) and from the InBio research group (Department of Biochemical and Microbial Technology, Ghent University, Belgium). This starting product consists of 93.4% C18:1 diacetylated subterminal hydroxylated sophorolipid lactone **1**, 4.1% diacetylated sophorolipid acids and 2.5% diacetylated terminal hydroxylated sophorolipid lactone. Small amounts of C18:0 and C18:2 lactone can be present in this fermentation product, together with the acidic forms due to hydrolysis. The vegetable oil of *Coriandrum sativum* fruits was isolated *via* twin-screw extrusion at LCA *via* a previously reported procedure.[121] Trimethylsulfonium hydroxide (TMSH), *t*-butyl methyl ether (TBME), hydrochloric acid (HCl), hexane, sodium citrate tribasic dihydrate, ammonium chloride (NH_4Cl), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), sodium, 4-dimethylaminopyridine (DMAP), Sudan III, diethyl ether, borane tetrahydrofuran complex (BH_3-THF), dibutylamine, *N*-butylmethylamine, *N*-butylbenzylamine, *N*-benzylmethylamine, *N*-methyloctadecylamine, *N*,*N*'-dimethylethylenediamine, *N*,*N*'-dimethylhexamethylenediamine, methylamine, isopropylamine, platinum black, acetic acid, acetyl chloride, potassium carbonate (K_2CO_3), acetonitrile, *N*-methylmorpholine *N*-oxide (NMMO), sodium borohydride ($NaBH_4$), oxalic acid, dimethyl carbonate, butyl iodide, sulfuric acid (H_2SO_4), iron(III) oxide (Fe_2O_3), 3-chloroperoxybenzoic acid (*m*CPBA), oleoyl chloride and hexamethylenediamine were purchased from Sigma-Aldrich (Belgium). Yeast extract was purchased from Difco (Belgium). Sodium hydroxide (NaOH), dry methanol, acetic anhydride, sodium triacetoxyborohydride ($NaBH(OAc)_3$), dimethylamine, diethylamine, dibenzylamine, butylamine, benzylamine, octadecylamine, sodium cyanoborohydride ($NaBH_3CN$), hydrogen peroxide, nonanal, triethylamine, dimethyl sulfide (Me_2S), pyridine, sodium bisulfite, sodium dodecyl sulfate, butyryl chloride, ethylenediamine and *o*-phenylenediamine were purchased from Acros Organics (Belgium). Magnesium sulfate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$) and methyl iodide were purchased from Fisher Scientific (Belgium). Octadecyl iodide, stearoyl chloride and octadecanal were purchased from TCI Europe (Belgium). Butyl bromide was purchased from De Bruyne Instruments (Belgium). Ethyl acetate, tetrahydrofuran (THF), ethanol and acetone were purchased from Chem-Lab (Belgium). Methanol and petroleum ether were purchased from VWR (Belgium). Glucose monohydrate was purchased from Cargill (Belgium). Sodium chloride (NaCl) was purchased from Colruyt (Belgium). Commercially available products were used without further purification. Dry tetrahydrofuran (THF) was distilled from sodium; dry dichloromethane was distilled from calcium hydride; dry acetonitrile

(ACN) was distilled over molecular sieves; dry ethanol (EtOH) was distilled from magnesium and iodine according to the method of Lund and Bjerrum.

4.1.2. Ozonolysis reaction

The ozonolysis reaction was performed with an Ozonia Triogen Model LAB2B laboratory ozone generator, connected to a Bronkhorst Flow-Bus E-7000 type mass flow meter to control the dry air inflow and an Anseros Ozomat Model GM Non-Dispersive UV-analyzer to measure the ozone concentration.

4.1.3. Chromatography purification of reaction mixtures

Analysis of reaction mixtures was performed *via* thin-layer chromatography (TLC) using silica plates (Merck Kieselgel 60 with F254 indicator, precoated, 0.25 mm) and different solvent mixtures. Visualization of the analyzed mixtures was performed by means of UV fluorescence (254 or 366 nm) or coloring with a potassium permanganate solution. Column chromatography was performed on silica gel (Acros, particle size 0.035-0.070 mm, pore diameter ca. 6 nm) or on alumina gel (Merck, pore size 60 Å, HF_{254}, type E, basic) in a glass column with different solvent mixtures. A suitable solvent system is determined *via* TLC analysis. Automated column chromatography is performed with a Grace Reveleris flash chromatography system with reusable silica columns (Reveleris, particle size 40-63 µm) as solid phase. The flow rate and gradient of the solvent system can be adjusted manually to the column size and the nature of the reaction mixture. Detection of the eluting compounds can be performed *via* UV analysis at two different wavelengths which can be chosen manually and *via* evaporative light-scattering detection (ELSD). Preparative TLC is performed with silica plates (Sigma-Aldrich, 2000 microns) as solid phase and different solvent mixtures. A suitable solvent system is determined *via* TLC analysis. Visualization of the compounds was performed by means of UV fluorescence (254 or 366 nm) or coloring with a potassium permanganate solution.

4.1.4. LC-MS chromatography

LC-MS analyses were performed on an Agilent apparatus, using a Supelco Ascentis Express C18 column (L 3 cm x ID 4.6 mm) with 2.7 µm fused-core particles (90 Å pore size). This apparatus is equipped with a UV detector (operating at 220.8 nm, 254.8 nm and 280.8 nm) and connected to an Agilent 1100 series LC/MSD type SL mass spectrometer with Electron Spray Ionization geometry (ESI 70 eV) and using a Mass Selective Detector (single quadrupole).

4.1.5. NMR spectroscopy

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance III Nanobay 400 MHz NMR spectrometer. The compounds were dissolved in deuterated solvents as indicated

with the spectral data of each compound. Tetramethylsilane (TMS) was used as an internal standard. The assignment of the different peaks was performed using DEPT, 2D-HSQC and 2D-COSY spectra. Peak multiplicities are described by the following abbreviations: s = singlet, d = doublet, t= triplet, q = quadruplet, sept = septet, m = multiplet, br = broad with their accompanying coupling constants.

4.1.6. Infrared spectroscopy

Infrared spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer. All compounds were analyzed in neat form with an Attenuated Total Reflectance (ATR) accessory while applied on a ZnSe crystal. Only selected absorbances υ_{max} are reported.

4.1.7. Mass analysis

Low resolution mass spectra of pure compounds were recorded *via* direct injection on an Agilent 1100 Series LC/MSD type SL mass spectrometer with Electron Spray Ionization geometry (ESI 70 eV) and using a Mass Selective Detector (quadrupole) with simultaneous positive and negative ion detection. High resolution mass spectrometry (HRMS) analyses were performed using an Agilent 1100 Series HPLC coupled to an Agilent 6220 TOF-Mass Spectrometer equipped with ESI/APCI-multimode source.

4.1.8. Melting points

Melting points of solid compounds were measured on a Wagner and Munz Kofler-Heizbank (Type WME) with a temperature range of 46-266 °C. Calibration of the apparatus was performed with reference materials.

4.1.9. Optical rotation

Optical rotation measurements are performed with a Jasco P-2000 Series polarimeter. A sample of the compound is dissolved in an optical cell (3.5 mm ID x 100 mm) at a well-known concentration c (mg/mL). The optical rotation is determined at a wavelength of 589 nm.

4.1.10. Determination of the fatty acid composition

Determination of the fatty acid composition of the coriander oil was performed by gas chromatography (GC). An oil sample was dissolved in TBME at a concentration of 20 mg/mL. A 100 µL aliquot of this solution was converted to methyl esters by the addition of 50 µL of a 0.2 M TMSH solution in methanol. The resulting fatty acid methyl esters were subjected to GC analysis using a Varian 3800 (USA) gas chromatograph equipped with a flame ionization detector. Separation of the methyl esters was achieved in a CP select CB (Varian, USA) fused silica capillary column (50 m, 0.25 mm i.d., 0.25 µm film thickness). The initial oven temperature was held at 185 °C for 40 minutes,

after which it was increased to 250 °C for 10 minutes. The temperature of the injector and the detector was kept at 250 °C. Helium was used as the carrier gas with a flow rate of 1.2 mL/min. The determination was carried out in triplicate.

4.1.11. Monitoring sophorolipid fermentation

Growth of the culture was frequently monitored by measuring optical density at 600 nm (Jasco, V-630 Bio Spectrophotometer). Cell dry weight was obtained by centrifugation (5 min, 14 000 rpm, Sigma 4-15 centrifuge) of 1 mL reactor broth in pre-dried and weighed falcons. The pellets were subsequently washed once with 1 mL physiological solution (9 g/L NaCl) and dried at 60 °C to a constant weight. Glucose concentrations were measured with a 2700 Select Biochemistry Analyzer (YSI).

4.1.12. Surface tension measurements

The determination of the surface tension was performed at room temperature *via* the Wilhelmy plate method. A platinum plate is brought into contact with an air-water surface and the force exerted on the plate is measured with a mass balance (Figure 36A). This force is directly related to the surface tension (Figure 36B). The surface tension is plotted in function of the concentration (log scale) and the CMC is determined as the lowest concentration for which the minimal surface tension is measured (Figure 36C). At increasing concentrations below the CMC, surfactant compounds assemble at the air-water interface until the interface is completely saturated with surfactant. Increasing the concentration above the CMC results in the formation of micelles.

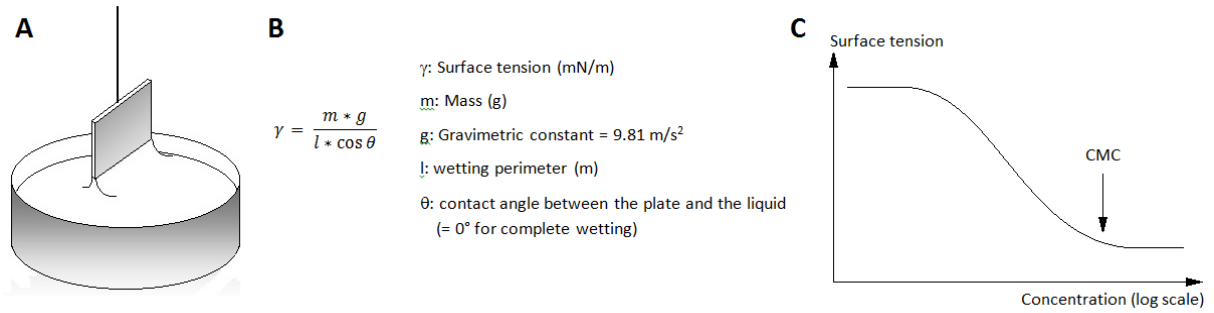


Figure 36. (A) Setup for surface tension measurements via the Wilhelmy plate method. (B) Formula to calculate the surface tension from the measured mass. (C) Surface tension in function of the concentration (log scale) to determine the critical micelle concentration.

All glassware and the platinum plate were rinsed with sulfochromic acid prior to the experiment to avoid interference of traces of residual compounds. A dilution series in distilled water ranging from 2.0 g/L to 0.01 mg/L was prepared and the surface tension was determined for each sample. The CMC values were determined as the concentration at which the minimal surface tension was reached when plotting the natural logarithm of the sophorolipid concentration against the surface tension.

For sophorolipid lactone **208**, the concentration of solubilized sophorolipid was determined with the TOC-5000 (Shimadzu) since this compound is not well soluble in water. With the bruto formula ($C_{34}H_{56}O_{14}$), the actual sophorolipid concentration could be calculated.

4.1.13. Antimicrobial activity assay

A first antimicrobial activity assay was performed at the Laboratory for Microbiology (Ghent University, Belgium). The six test strains *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095, *Staphyllococcus aureus* LMG 8064, *Bacillus subtilis* LMG 13579, *Enterococcus faecium* LMG 11397 and *Streptococcus pneumoniae* LMG 16738 were grown in Mueller Hinton II (MH) broth medium. LMG 13579 was incubated at 28 °C for 24 h, all other strains were incubated at 37 °C. Inocula with a density of 10^5 CFU/mL were prepared in MH broth. The bioassay was carried out in sterile 96-well microtiter plates. The test compounds were delivered as a solution in DMSO at a concentration of approximately 50 mg/mL. For the antimicrobial screening, working solutions of test compounds were prepared by mixing 200 µL of the supplied DMSO solutions of the compounds and 800 µL MH culture broth. The antimicrobial activity of each compound was tested in duplicate against a single test strain in a test volume of 200 µL at a final concentration of approximately 0.5 mg/mL test compound and 10^4 CFU/mL test bacteria. Positive and negative control consisted of a 0.5 mg/mL gentamicin sulphate solution and sterile saline, respectively. For the determination of the MIC values, a stock solution of 2 mg/mL was used to prepare the dilution series. The MIC of the selected compounds was tested in duplicate against a single test strain in a test volume of 200 µL at a final concentration ranging from 100 to 2.5 µg/mL test compound and 10^4 CFU/mL test bacteria. For comparative purposes, a dilution series of the antibiotic gentamicin sulfate was included in a concentration ranging from 100 to 2.5 µg/mL. Depending on the test strains, plates were incubated at 28 or 37 °C for 24 h under aerobic conditions. Bacterial growth was scored visually. Turbidity levels comparable to those in the positive or negative control wells were regarded as positive or negative, respectively, for antimicrobial activity of the compound in question.

A second antimicrobial activity assay was performed at the Laboratory for Microbiology (Ghent University). Antimicrobial activity against *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095, *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 was assessed by a broth dilution method (CLSI, 2012). Strains with LMG designation were obtained from the BCCM/LMG Bacteria Collection (Ghent, Belgium) while strain ATCC 6538 was obtained from the American Type Culture Collection (Manassas, VA). *S. aureus* Mu50 was a kind gift of P. Vandamme (Ghent, Belgium). All strains were grown aerobically at 37 °C on Mueller Hinton agar (LabM, Heywood, UK). The minimal inhibitory concentration that inhibited growth by at least 50%

compared to the untreated control ($MIC_{1/2}$), the minimal inhibitory concentration that inhibited growth completely (MIC) and the minimal bactericidal concentration at which no more cell viability of the test organism can be observed (MBC) were used as a measure of activity. $MIC_{1/2}$, MIC and MBC values were determined using flat-bottomed 96-well microtiter plates (TPP, Trasadingen, Switzerland). Concentrations of compounds tested ranged from 0.48 to 2500 µg/mL in Mueller Hinton broth (LabM). The inoculum was standardized at approximately 5 x 10^5 colony forming units/mL. The plates were incubated at 37 °C for 24 h and the optical density was determined at 590 nm using a multilabel microtiter plate reader (Envision Xcite, Perkin Elmer LAS, Waltham, MA).

Clinical and Laboratory Standards Institute (CLSI) (2012) Performance Standards for Antimicrobial Susceptibility Testing. Twentieth second Informational Supplement M100-S22. Wayne, PA, USA.

The biofilm assay was performed at the Laboratory for Microbiology (Ghent University). *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50 were cultured on Trypton Soy agar plates (TSA, LabM, Lancashire, UK). From these pure cultures, overnight suspensions were made by inoculating 10 mL Trypton Soy broth (TSB, LabM, Lancashire, UK) with a loopful of microorganisms. Both strains were grown aerobically at 37 °C. Biofilms were formed as previously described.[180] Overnight suspensions were adjusted with TSB of 0.05. These optical densities correspond approximately 2.5 x 10^7 Colony Forming Units (CFU)/mL. A 100 µL of the diluted cell suspension were transferred to the wells of a polystyrene round-bottomed 96-well microtiter plate (MTP, SPL, Lifescience, Korea) and incubated at 37 °C. Blanco control wells were filled with sterile medium. Medium was removed after 4 h and biofilms were rinsed with Physiological Saline (PS) to remove non-adhered cells. Fresh medium was subsequently added to the wells and plates were further incubated for 20 h. These biofilms were rinsed with PS and treated with 100 µL of test compound at a concentration of 20 µg/mL for 24 h at 37 °C. The treatments were removed and the biofilms were rinsed with PS. To determine the CFU, 100 µL PS was added to the wells containing the treated biofilms and the MTP was sonicated and vortexed twice. The detached cells were quantified by conventional plating.

4.1.14. SAXS analysis

In small-angle X-ray scattering (SAXS) experiments, the scattering of a monochromatic X-ray beam over a certain range of scattering angles upon contact with a sample is analyzed (Figure 37A). For small scattering angles, q-values are calculated in function of the scattering angle and the wavelength of the X-ray beam (Figure 37B). An isotropic scattering pattern is detected on the X-ray detector, displaying a variety in scattering intensity in function of the scattering angles (Figure 37C). A plot is made displaying the integrated scattering intensity over all scattering angles in function of

the q-value (Figure 37D). This plot contains information on the size and shape of the particles present in the analyzed sample. To determine the particle size, a Guinier plot is made in which q^2 is plotted in function of log[I(q)]. From the slope of the obtained graph in the low q-region, the radius of gyration is derived which has a direct relationship to the radius of the particles.

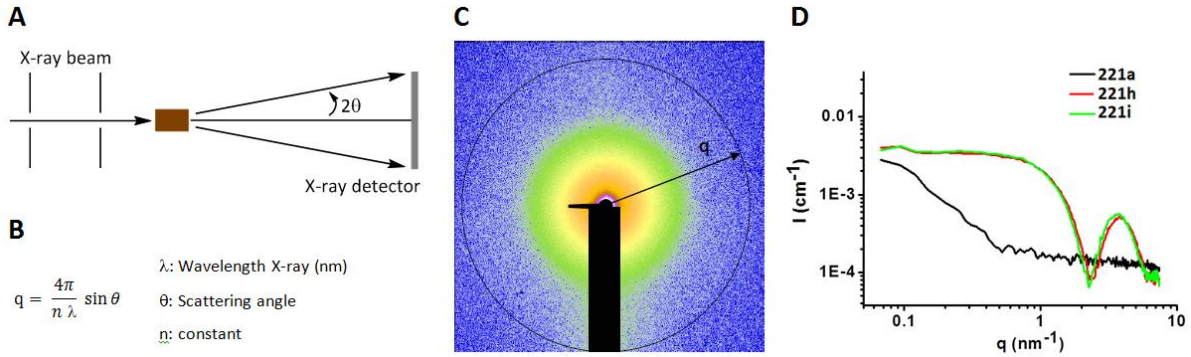


Figure 37. (A) Scattering of a monochromatic X-ray beam over a certain range of scattering angles. (B) Formula to calculate the q-value. (C) Isotropic scattering pattern detected with the X-ray detector. (D) Small-angle X-ray scattering (SAXS) plot displaying the scattering intensity I(q) in function of the q-value.

SAXS experiments have been performed using a flow-through polycarbonate 2 mm capillary. Data have been acquired on the high brilliance ID02 beamline at the ESRF synchrotron (Grenoble, France) using a 1 m sample-to-detector distance and an energy of 12.46 keV. The acquisition time was 100 ms and three experiments were averaged out. A CCD camera was used to collect the scattered photons and integrated azimuthally to obtain a typical I(q) spectrum. Contribution of the solvent (water) and capillary have been measured prior to the experiment and were duly subtracted during the data treatment. All data have been corrected for the transmission of the direct beam and were scaled to be in absolute scale.

4.1.15. Transfection assay

The liposomal solutions were prepared by hydration of a lipid film. A 1.5 mM solution (1 mL) of each compound was prepared in chloroform, formulated with or without DOPE (1:1 compound/DOPE) and evaporated under reduced pressure to produce a thin lipid film. Water (1 mL) was added to rehydrate this lipid film in a time period of 7 days at room temperature. The solution was vortexed (10 s) and sonicated (30 min at 50 °C) at 45 kHz using a VWR ultrasonic bath. The size and zeta potential were determined for each liposomal formulation.

Lipoplexes were prepared by mixing pDNA (pEGFP-Luc, Clontech) with each liposomal solution in OptiMEM (Gibco). Addition of pDNA to the liposomal solutions was performed at concentrations corresponding to CR ranging from 1 to 8. The obtained mixtures were incubated at room temperature for 1 hour before being subjected to electrophoresis in a 0.8% agarose gel at 100 V,

90 mA. The gel was previously stained with SYBRgold nucleic acid gel staining (Life Technologies) and visualized using a UV transilluminator (Fischer Bioblock).

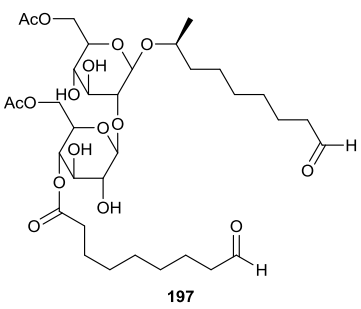
The *in vitro* reporter gene assay *via* luciferase measurement was carried out as reported previously.[150, 181] In short, the four cell lines were grown in either EMEM (16HBE) or DMEM (A549, SKMEL28 and C2C12) both supplemented with 10% bovine fetal serum, 1% antibiotic and 1% *L*-glutamine. All incubations were performed at 37 °C in a humidified atmosphere containing 5% CO_2. The day before transfection, the cells were seeded into a 96-well plate at a density of 25,000 cells per well. Lipoplexes were prepared as detailed above and then added dropwise to each well; the commercial formulation Lipofectamine Reagent (Invitrogen) was used as a positive transfection control whereas naked DNA was used as negative control. DOPE was not added to the positive and negative control. After 48 h at 37 °C, the culture medium was removed and the cells were lysed with Passive Lysis Buffer (Promega) prior to examination *via* a chemiluminescent assay (Luciferase Assay System, Promega) to determine the luciferase expression. The total protein content of each cell lysate was determined using the BC assay kit (Uptima). Finally, data were expressed as relative light units (RLU) per milligram of total proteins (mean ± SD with n=3).

The cell viability was considered as a marker of the toxicity resulting from the exposition of the cells to the lipoplexes. For this purpose, the Vialight kit (Lonza) was used to determine the ATP content which reflects the number of living cells in culture. This assay was used as recommended by the manufacturer. Results were expressed as percentages relative to the viability of non-transfected cells used as reference (100% cell viability).

4.2. Synthetic procedures and characterization

4.2.1. Synthesis of the intermediate sophorolipid aldehyde

General procedure for the synthesis of *(S)*-8-([4"-(9-oxononanoyl)oxy,6',6"-diacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-nonanal (197): In a 250 mL flame dried washing flask, 8.04 g sophorolipid lactone **1** (11.67 mmol, 1 eq) was dissolved in 100 mL dry CH_2Cl_2 and a pinch of Sudan III was added. The reaction mixture was cooled down to -78 °C and sparged with ozone until the red color of the reaction mixture dissipated. Upon the addition of 4.35 g Me_2S (70.03 mmol, 6eq), the mixture was stirred for 1 h at room temperature. The mixture was washed 3 times with 10 mL brine and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Sophorolipid dialdehyde **197** was purified by automated column chromatography as an off-white solid with a petroleum ether/ethyl acetate mixture as eluent (2.00 g, 24%). Gradient: 2 CV 20% EtOAc, 25 CV 20-100% EtOAc, 13 CV 100% EtOAc.



197

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 1.23 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.26-1.46 (13H, m, C$\underline{H}_a$H$_b$CHCH$_3$, 6xC$\underline{H}_2$(CH$_2$)$_2$), 1.56-1.66 (7H, m, CH$_a\underline{H}_b$CHCH$_3$, C$\underline{H}_2$CH$_2$(C=O)O, 2xC$\underline{H}_2$CH$_2$(C=O)H), 2.07 (3H, s, C$\underline{H}_3$C=O), 2.09 (3H, s, C$\underline{H}_3$C=O), 3.32-2.37 (2H, m, CH$_2$C$\underline{H}_2$(C=O)O), 2.43 (2H, txd, J=7.2 Hz, J=1.6 Hz, C$\underline{H}_2$(C=O)H), 2.45 (2H, txd, J=7.2 Hz, J=1.6 Hz, C$\underline{H}_2$(C=O)H), 3.39-3.57 (4H, m, 4xC$\underline{H}$OC), 3.62-3.70 (3H, m, 3x C$\underline{H}$OC), 3.73-3.80 (1H, m, CH$_3$C$\underline{H}$O), 4.08-4.12 (1H, m, CHC$\underline{H}_a$H$_b$OAc), 4.19 (1H, dxd, *J*=12.2 Hz, *J*=4.9 Hz, CHCH$_a\underline{H}_b$OAc), 4.31-4.35 (2H, m, CHC$\underline{H}_2$OAc), 4.45 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.54 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$), 4.91 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C$\underline{H}$OC), 5.06 (4H, br s, 4xO$\underline{H}$), 9.75 (1H, t, J=1.7 Hz, CH$_2$(C=O)$\underline{H}$), 9.76 (1H, t, J=1.6 Hz, CH$_2$(C=O)$\underline{H}$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.8 (C$\underline{H}_3$C=O), 20.9 (C$\underline{H}_3$C=O), 21.3 (C$\underline{H}_3$CH), 21.9 (C$\underline{H}_2$CH$_2$(C=O)H), 21.9 (C$\underline{H}_2$CH$_2$(C=O)H), 24.6 (C$\underline{H}_2$CH$_2$(C=O)O), 24.9 (C$\underline{H}_2$(CH$_2$)$_2$), 28.7 (C$\underline{H}_2$(CH$_2$)$_2$), 28.8 (C$\underline{H}_2$(CH$_2$)$_2$), 28.9 (C$\underline{H}_2$(CH$_2$)$_2$), 29.1 (C$\underline{H}_2$(CH$_2$)$_2$), 29.4 (C$\underline{H}_2$(CH$_2$)$_2$), 34.1 (CH$_2$C$\underline{H}_2$(C=O)O), 36.4 (C$\underline{H}_2$CHCH$_3$), 43.8 (C$\underline{H}_2$(C=O)H), 43.8 (C$\underline{H}_2$(C=O)H), 62.4 (C$\underline{H}_2$OAc), 63.5 (C$\underline{H}_2$OAc), 69.8 (C$\underline{H}$OC), 70.4 (C$\underline{H}$OC), 72.6 (C$\underline{H}$OC), 73.3 (C$\underline{H}$OC), 74.0 (C$\underline{H}$OC), 74.1 (C$\underline{H}$OC), 76.0 (C$\underline{H}$OC), 77.4 (CH$_3$C$\underline{H}$O), 81.5 (C$\underline{H}$OC), 100.9 (C$\underline{H}$(O)$_2$), 103.6 (C$\underline{H}$(O)$_2$), 170.7 (CH$_2$(C=O)O), 171.5 (CH$_2$(C=O)O), 173.2 (CH$_2$(C=O)O), 203.0 (CH$_2$(C=O)H), 203.1 (CH$_2$(C=O)H).

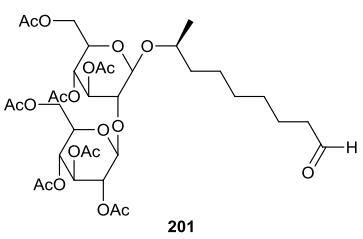
General procedure for the synthesis of methyl *(S)*-17-([2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (89a): In a 100 mL flame dried round-bottomed flask, 29.80 g sophorolipid lactone **1** (43.27 mmol, 1 eq) was dissolved in 50 mL dry methanol and 1.62 mL sodium methoxide (6.49 mmol, 0.15 eq) was added. The flask was equipped with a reflux condenser and a $CaCl_2$ guard-tube to protect the reaction mixture from atmospheric moisture. The reaction

mixture was stirred for 3h at reflux temperature, cooled down to room temperature and acidified to neutral pH with acetic acid. The mixture was concentrated under reduced pressure, dissolved in deionized water and cooled down to 0 °C in an ice bath. The sophorolipid methyl ester **89a** precipitated as a white powder. The precipitate was filtered, washed with water and dried under reduced pressure (22.26 g, yield 81%).

General procedure for the synthesis of methyl *(S,Z)*-17-([2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-9-octadecenoate (114a): In a 100 mL flame dried round-bottomed flask, 21.56 g sophorolipid methyl ester **89a** (33.86 mmol, 1 eq) was dissolved in 100 mL dry THF. The flask was equipped with a $CaCl_2$ guard-tube to protect the reaction mixture from atmospheric moisture and 25.4 mL acetic anhydride (270.84 mmol, 8 eq) and 1.654 g DMAP (13.54 mmol, 0.4 eq) were added. The reaction mixture was stirred for 1 h at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a 10 mL saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Peracetylated sophorolipid methyl ester **114a** was isolated as a viscous colorless oil (32.49 g, quantitative yield).

General procedure for the synthesis of *(S)*-8-([2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-nonanal (201): In a 250 mL flame dried washing flask, 12.49 g peracetylated sophorolipid methyl ester **114a** (13.41 mmol, 1 eq) was dissolved in 100 mL methanol. A pinch of Sudan III indicator and 1 equivalent of $NaHCO_3$ were added. The reaction mixture was sparged with ozone at room temperature until the red color of the reaction mixture dissipated. The mixture was stirred for 1 h at room temperature with 2.84 g $NaBH(OAc)_3$ (13.41 mmol, 1 eq), concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with 10 mL brine and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Peracetylated sophorolipid aldehyde **201** was purified by automated column chromatography as a viscous colorless oil with a petroleum ether/diethyl ether mixture as eluent (7.03 g, 67%). Gradient: 2CV 20% Et_2O, 15 CV 20-100% Et_2O, 8 CV 100% Et_2O.

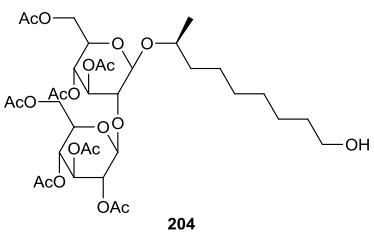
For characterization purposes, a small amount of sophorolipid aldehyde **201** was purified through a sodium bisulfite addition reaction. In a 50 mL round-bottomed flask, 0.42 g sophorolipid aldehyde is dissolved in 25 mL ethyl acetate and $NaHSO_3$ (2.67 mmol, 5 eq) was added. The mixture was stirred for 30 min at room temperature, extraxted 3 times with 10 mL water and 2.34 mL saturated $NaHCO_3$-solution (2.67 mmol, 5 eq) were added to the water phase. The water phase was extracted 3 times with 10 mL ethyl acetate and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure.



201

IR (cm^{-1}) υ_{max}: 1036 (CHOCH), 1064 (CHOCH), 1224 (COAc), 1367, 1748 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.28-1.45 (7H, m, 3xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.54-1.62 (1H, m, CH$_a$$\underline{H}_b$CHCH$_3$), 1.68 (2H, txt, *J*=7.4 Hz, *J*=7.3 Hz, C$\underline{H}_2$CH$_2$(C=O)H), 1.99 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.02 (3H, s, C$\underline{H}_3$C=O), 2.03 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (3H, s, C$\underline{H}_3$C=O), 2.09 (3H, s, C$\underline{H}_3$C=O), 2.47 (2H, txd, *J*=7.4 Hz, J=1.8 Hz, C$\underline{H}_2$(C=O)H), 3.63-3.76 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.06-4.11 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.23-4.31 (2H, m, 2xCHCH$_a$$\underline{H}_b$OAc), 4.46 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.71 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.90 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, C$\underline{H}$OC), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, C$\underline{H}$OC), 5.05 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C$\underline{H}$OC), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, C$\underline{H}$OC), 5.17 ((1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 9.78 (1H, t, *J*=1.8 Hz, $\underline{H}$C=O). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.3 (C$\underline{H}_3$C=O), 20.4 (2xC$\underline{H}_3$C=O), 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.7 (C$\underline{H}_3$C=O), 21.3 (C$\underline{H}_3$CH), 22.1 (C$\underline{H}_2$CH$_2$(C=O)H), 24.6 (C$\underline{H}_2$(CH$_2$)$_2$), 29.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.4 (C$\underline{H}_2$(CH$_2$)$_2$), 36.4 (C$\underline{H}_2$CHCH$_3$), 43.7 (C$\underline{H}_2$(C=O)H), 61.9 (C$\underline{H}_2$OAc), 62.1 (C$\underline{H}_2$OAc), 68.2 (C$\underline{H}$OC), 68.7 (C$\underline{H}$OC), 71.2 (C$\underline{H}$OC), 71.5 (C$\underline{H}$OC), 71.6 (C$\underline{H}$OC), 72.9 (C$\underline{H}$OC), 74.6 (C$\underline{H}$OC), 77.3 (C$\underline{H}$OC), 77.9 (C$\underline{H}$OC), 100.3 (C$\underline{H}$(O)$_2$), 101.2 (C$\underline{H}$(O)$_2$), 169.3 (CH$_3$C=O), 169.4 (CH$_3$C=O), 169.6 (CH$_3$C=O), 169.9 (CH$_3$C=O), 170.1 (CH$_3$C=O), 170.4 (CH$_3$C=O), 170.5 (CH$_3$C=O), 202.9 (HC=O). **MS (ESI): m/z** Exact mass calculated for $C_{35}H_{56}NO_{19}$ [M+NH_4^+]: 794.3441. Found: 794.3438.

General procedure for the synthesis of *(S)*-8-([2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl]-oxy)-nonanol (204): In a 250 mL flame dried washing flask, 2.12 g peracetylated sophorolipid methyl ester **114a** (2.28 mmol, 1 eq) was dissolved in 100 mL dry CH_2Cl_2. A pinch of Sudan III indicator and 0.07 g methanol (2.28 mmol, 1 eq) were added. The reaction mixture was sparged with ozone at room temperature until the red color of the reaction mixture dissipated. The mixture was stirred for 1 h at room temperature with 0.22 g $NaBH_4$ (5.70 mmol, 2.5 eq). The mixture was washed 3 times with 10 mL brine and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Peracetylated sophorolipid alcohol **204** was purified by automated column chromatography as a viscous colorless oil with a petroleum ether/ethyl acetate mixture as eluent (1.02 g, 58%). Gradient: 10 CV 20% EtOAc, 10 CV 30% EtOAc, 20 CV 40% EtOAc, 5 CV 80% EtOAc, 5 CV 90% EtOAc.

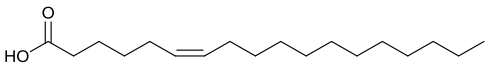


204

IR (cm^{-1}) υ_{max}: 1034(CHOCH), 1063 (CHOCH), 1215 (COAc), 1366, 1744 (C=O), 3540 (OH). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.26-1.46 (9H, m, 4xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.54-1.65 (3H, m, CH$_a$$\underline{H}_b$CHCH$_3$, C$\underline{H}_2CH_2$OH), 1.99 (3H, s, C$\underline{H}_3$C=O),

2.00 (3H, s, CH_3C=O), 2.02 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.09 (6H, s, 2xCH_3C=O), 3.62-3.75 (6H, m, 2xCHCH_2OAc, CH_3CHO, CHOC, CH_2OH), 4.07-4.11 (2H, m, 2xCHCH_aH$_b$OAc), 4.22-4.32 (2H, m, 2xCHCH$_a$H$_b$OAc), 4.46 (1H, d, *J*=7.6 Hz, CH(O)$_2$), 4.71 (1H, d, *J*=8.0 Hz, CH(O)$_2$), 4.91 (1H, dxd, *J*=9.5 Hz, *J*=6.4 Hz, CHOC), 4.93 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, CHOC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, CHOC), 5.17 ((1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 20.4 (CH_3C=O), 20.5 (CH_3C=O), 20.6 (2xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.5 (CH_3CH), 24.9 (CH_2(CH$_2$)$_2$), 25.7 (CH_2(CH$_2$)$_2$), 29.7 (CH_2(CH$_2$)$_2$), 29.7 (CH_2(CH$_2$)$_2$), 32.8 (CH_2CH$_2$OH), 36.7 (CH_2CHCH$_3$), 62.0 (CH_2OAc), 62.3 (CH_2OAc), 62.7 (CH_2OH), 68.3 (CHOC), 68.8 (CHOC), 71.3 (CHOC), 71.6 (CHOC), 71.7 (CHOC), 73.0 (CHOC), 74.8 (CHOC), 77.5 (CHOC), 78.2 (CHOC), 100.4 (CHOC), 101.4 (CHOC), 169.5 (CH$_3$C=O), 169.6 (CH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.3 (CH$_3$C=O), 170.6 (2xCH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{35}H_{58}NO_{19}Na$ [M+NH_4^+]: 796.3598. Found: 796.3588.

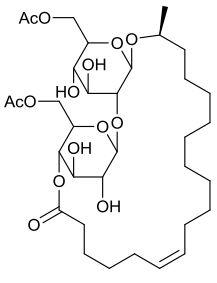
General procedure for the isolation of petroselinic acid (207): In a 2 L flask, 446.10 g of coriander oil is weighed and 800 ml of a 3N sodium hydroxide solution is added. The reaction mixture is refluxed for 2.5 hours, cooled down and acidified to pH 1 with a 3 N hydrochloric acid solution. The mixture is extracted with hexane and washed with water. The organic phase is dried over magnesium sulfate, filtered and concentrated under reduced pressure. Pure petroselinic acid is obtained through crystallization in absolute ethanol at -20 °C as a white solid at room temperature (251.07 g, 80%).



207

^{1}H-NMR (400 MHz, CDCl$_3$): δ_H 0.88 (3H, t, *J*=6.8 Hz, CH_3), 1.26-1.36 (18H, m, 9xCH_2), 1.37-1.44 (2H, m, CH_2(CH$_2$)$_2$COOH), 1.62-1.69 (2H, m, CH_2CH$_2$COOH), 2.01 (2H, dxt, *J*=7.0 Hz, *J*=6.9 Hz, CH_2CH=CH), 2.05 (2H, dxt, J=7.1 Hz, J=7.1 Hz, CH_2CH=CH), 2.36 (2H, t, *J*=7.5 Hz, CH_2COOH), 5.29-5.41 (2H, m, CH=CH), 11.60 (1H, br s, COOH). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 14.1 (CH_3), 22.7 (CH_2), 24.3 (CH_2CH$_2$COOH), 26.8 (CH_2CH=CH), 27.2 (CH_2CH=CH), 29.1 (CH_2(CH$_2$)$_2$COOH), 29.3, 29.4, 29.6, 29.7, 29.7, 29.7, 31.9 (8xCH_2), 34.0 (CH_2COOH), 128.9 (CH=CH), 130.6 (CH=CH), 180.1 (COOH). **T_m:** 30-31°C.

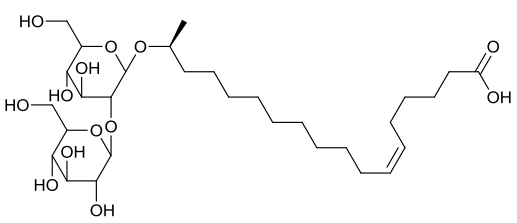
Characterization of petroselinic acid based diacetylated sophorolipid lactone (208): ^{1}H-NMR (400 MHz, MeOD): δ_H 1.23 (3H, d, *J*=6.2 Hz, CHCH_3), 1.31-1.50 (16H, m, 7xCH$_2$(CH$_2$)$_2$, CH_aH$_b$CHCH$_3$, CH_aH$_b$CH$_2$CHCH$_3$), 1.53-1.59 (2H, m, CH$_a$H$_b$CHCH$_3$, CH$_a$H$_b$CH$_2$CHCH$_3$), 1.61-1.74 (2H, m, CH_2CH$_2$C=O), 2.03-2.11 (4H, m, 2xCH_2CH=CH), 2.06 (3H, s, CH_3C=O), 2.07 (3H, s, CH_3C=O), 2.33-2.47 (2H, m, CH_2C=O), 3.29-3.36 (2H, m, 2xCHOC), 3.45 (1H, dxd, *J*=9.1 Hz, *J*=7.7 Hz, CHOC), 3.46-3.50 (1H, m, CHOC), 3.58 (1H, dxd, *J*=9.1 Hz, *J*=9.1 Hz, CHOC), 3.60 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 3.66 (1H, dxdxd, *J*=10.0 Hz, *J*=3.8 Hz, *J*=3.8 Hz, CHOC), 3.71-3.79 (1H, m, CH$_3$CHO), 4.09-4.14



208

(2H, m, C$\underline{H}_2$OAc), 4.21 (1H, dxd, *J*=11.8 Hz, *J*=6.4 Hz, C$\underline{H}_a$H$_b$OAc), 4.38 (1H, dxd, *J*=11.8 Hz, *J*=2.1 Hz, CH$_a\underline{H}_b$OAc),4.46 (1H, d, *J*=7.6 Hz, CH(O)$_2$), 4.67 (1H, dxd, *J*=7.7 Hz, CH(O)$_2$), 4.84-4.89 (1H, m, C$\underline{H}$OC), 5.33-5.43 (2H, m, C$\underline{H}$=C$\underline{H}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 19.3 (C$\underline{H}_3$C=O), 19.5 (C$\underline{H}_3$C=O), 20.5 (C$\underline{H}_3$CH), 23.9 (C$\underline{H}_2$CH$_2$C=O), 25.1 (C$\underline{H}_2$CH$_2$CHCH$_3$), 26.1 (C$\underline{H}_2$CH=CH), 26.6 (C$\underline{H}_2$CH=CH), 28.5 (C$\underline{H}_2$(CH$_2$)$_2$), 29.0 (C$\underline{H}_2$(CH$_2$)$_2$), 29.2 (C$\underline{H}_2$(CH$_2$)$_2$), 29.7 (C$\underline{H}_2$(CH$_2$)$_2$), 29.7 (C$\underline{H}_2$(CH$_2$)$_2$), 29.8 (C$\underline{H}_2$(CH$_2$)$_2$), 30.0 (C$\underline{H}_2$(CH$_2$)$_2$), 33.2 (C$\underline{H}_2$C=O), 37.2 (C$\underline{H}_2$CHCH$_3$), 62.7 (C$\underline{H}_2$OAc), 63.4 (C$\underline{H}_2$OAc), 70.2 (C$\underline{H}$OC), 70.5 (C$\underline{H}$OC), 72.0 (C$\underline{H}$OC), 73.4 (C$\underline{H}$OC), 73.6 (C$\underline{H}$OC), 75.2 (C$\underline{H}$OC), 76.5 (C$\underline{H}$OC), 78.7 (C$\underline{H}$OC), 81.9 (C$\underline{H}$OC), 102.2 (C$\underline{H}$(O)$_2$), 103.8 (C$\underline{H}$(O)$_2$), 128.6 (C$\underline{H}$=CH), 130.5 (C$\underline{H}$=CH), 170.8 (C$\underline{=}$O), 171.3 (C$\underline{=}$O), 172.9 (C$\underline{=}$O).

General procedure for the synthesis of *(S,Z)*-17-([2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-6-octadecenoic acid (209): In a 100 mL round-bottomed flask, 1.01 g lactonic SL **208** (1.47 mmol, 1 eq) was dissolved in aqueous 3 N NaOH and refluxed for 20 minutes. The reaction mixture was cooled down and acidified with concentrated HCl to pH 5 to induce precipitation of sophorolipid acid **209** as a white powder. The precipitate was filtered, washed with water and dried under reduced pressure (0.87 g, yield 95%).

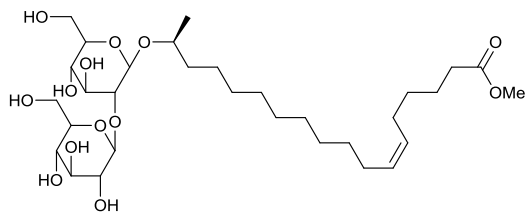


209

^{1}H-NMR (400 MHz, MeOD): δ_H 1.27 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.30-1.48 (17H, m, 8xCH$_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.60-1.67 (3H, m, CH$_a\underline{H}_b$CHCH$_3$, C$\underline{H}_2$CH$_2$COOH), 2.04-2.11 (4H, m, 2xC$\underline{H}_2$CH=CH), 2.31 (2H, t, *J*=7.4 Hz, C$\underline{H}_2$COOH), 3.24-3.36 (5H, m, C$\underline{H}$OC), 3.40 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, C$\underline{H}$OC), 3.47 (1H, dxd, *J*=8.4 Hz, *J*=8.4 Hz, C$\underline{H}$OC), 3.58 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, C$\underline{H}$OC), 3.66-3.71 (2H, m, 2xC$\underline{H}_a$H$_b$OH), 3.82-3.89 (3H, m, 2xCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.47 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.66 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$), 5.33-5.43 (2H, m, C$\underline{H}$=C$\underline{H}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.5 (C$\underline{H}_3$CH), 24.3 (C$\underline{H}_2$CH$_2$COOH), 24.9 (C$\underline{H}_2$(CH$_2$)$_2$), 26.4 (C$\underline{H}_2$CH=CH), 26.8 (C$\underline{H}_2$CH=CH), 28.9 (C$\underline{H}_2$(CH$_2$)$_2$), 29.0 (C$\underline{H}_2$(CH$_2$)$_2$), 29.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.5 (3xC$\underline{H}_2$(CH$_2$)$_2$), 29.6 (C$\underline{H}_2$(CH$_2$)$_2$), 33.5 (C$\underline{H}_2$COOH), 36.4 (C$\underline{H}_2$CHCH$_3$), 61.3 (C$\underline{H}_2$OH), 61.6 (C$\underline{H}_2$OH), 70.1 (C$\underline{H}$OC), 70.4 (C$\underline{H}$OC), 74.4 (C$\underline{H}$OC), 76.3 (2xC$\underline{H}$OC), 76.8 (C$\underline{H}$OC), 76.9 (C$\underline{H}$OC), 77.5 (C$\underline{H}$OC), 80.5 (C$\underline{H}$OC), 101.3 (C$\underline{H}$(O)$_2$), 103.2 (C$\underline{H}$(O)$_2$), 128.9 (C$\underline{H}$=CH), 129.9 (C$\underline{H}$=CH), 176.2 (C$\underline{O}$OH).

General procedure for synthesis of methyl *(S,Z)*-17-([2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl]-oxy)-6-octadecenoate (210): In a 100 mL flame dried round-bottomed flask, sodium methoxide was formed *in situ* by addition of 0.05 g sodium (2.05 mmol, 0.15 eq) to 20 mL dry methanol and 9.39 g lactonic SL **208** (13.64 mmol, 1 eq) was subsequently added. The flask was equipped with a reflux condenser and a $CaCl_2$ guard-tube to protect the reaction mixture from atmospheric moisture. The reaction mixture was stirred for 3 h at reflux temperature, cooled down to room temperature and

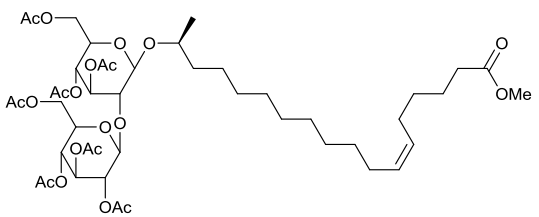
acidified to neutral pH with acetic acid. The mixture was concentrated under reduced pressure, dissolved in deionized water and cooled down to 0 °C in an ice bath. The sophorolipid methyl ester **210** precipitated as a white powder. The precipitate was filtered, washed with water and dried under reduced pressure (7.81 g, yield 90%).



210

^{1}H-NMR (400 MHz, MeOD): δ_H 1.27 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.31-1.50 (17H, m, C$\underline{H}_a$H$_b$CHCH$_3$, 8xC$\underline{H}_2$(CH$_2$)$_2$), 1.60-1.67 (3H, m, CH$_a\underline{H}_b$CHCH$_3$, C$\underline{H}_2$CH$_2$COOMe), 2.03-2.10 (4H, m, 2xC$\underline{H}_2$CH=CH), 2.34 (2H, t, *J*=7.4 Hz, C$\underline{H}_2$COOMe), 3.23-3.36 (5H, m, 5xC$\underline{H}$OC), 3.40 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, C$\underline{H}$OC), 3.45-3.49 (1H, m, C$\underline{H}$OC), 3.58 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, CHOC), 3.65-3.70 (2H, m, 2xC$\underline{H}_a$H$_b$OH), 3.67 (3H, s, OC$\underline{H}_3$), 3.81-3.89 (3H, m, 2xCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.47 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.66 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$), 5.32-5.43 (2H, m, C$\underline{H}$=C$\underline{H}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.5 (C$\underline{H}_3$CH), 24.2 (C$\underline{H}_2$CH$_2$COOMe), 24.9 (C$\underline{H}_2$(CH$_2$)$_2$), 26.4 (C$\underline{H}_2$CH=CH), 26.8 (C$\underline{H}_2$CH=CH), 28.9 (C$\underline{H}_2$(CH$_2$)$_2$), 29.0 (C$\underline{H}_2$(CH$_2$)$_2$), 29.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.5 (3xC$\underline{H}_2$(CH$_2$)$_2$), 29.6 (C$\underline{H}_2$(CH$_2$)$_2$), 33.3 (C$\underline{H}_2$COOMe), 36.5 (C$\underline{H}_2$CHCH$_3$), 50.7 (OC$\underline{H}_3$), 61.4 (C$\underline{H}_2$OH), 61.7 (C$\underline{H}_2$OH), 70.1 (C$\underline{H}$OC), 70.4 (C$\underline{H}$OC), 74.5 (C$\underline{H}$OC), 76.4 (2xC$\underline{H}$OC), 76.8 (C$\underline{H}$OC), 76.9 (C$\underline{H}$OC), 77.5 (C$\underline{H}$OC), 80.5 (C$\underline{H}$OC), 101.3 (C$\underline{H}$(O)$_2$), 103.3 (C$\underline{H}$(O)$_2$), 128.9 (C$\underline{H}$=CH), 130.0 (C$\underline{H}$=CH), 174.5 (C$\underline{O}$OMe).

General procedure for synthesis of methyl *(S,Z)*-17-([2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl]-oxy)-6-octadecenoate (211): In a 100 mL flame dried round-bottomed flask, 5.59 g sophorolipid methyl ester **210** (8.78 mmol, 1 eq) was dissolved in 50 mL dry THF. The flask was equipped with a $CaCl_2$ guard-tube to protect the reaction mixture from atmospheric moisture and 5.93 mL acetic anhydride (61.87 mmol, 7.05 eq) and 0.43 g DMAP (3.51 mmol, 0.4 eq) were added. The reaction mixture was stirred for 1 h at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a 10 mL saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Peracetylated sophorolipid methyl ester **211** was isolated as a viscous colorless oil (8.17 g, quantitative yield).

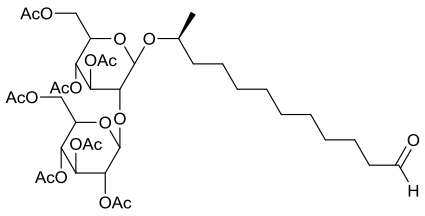


211

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.26-1.41 (17H, m, C$\underline{H}_a$H$_b$CHCH$_3$, 8xC$\underline{H}_2$(CH$_2$)$_2$), 1.56-1.68 (3H, m, CH$_a\underline{H}_b$CHCH$_3$, C$\underline{H}_2$CH$_2$COOMe), 1.98-2.08 (4H, m, 2xC$\underline{H}_2$CH=CH), 1.98 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.01 (3H, s, C$\underline{H}_3$C=O), 2.03 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.31 (2H, t, *J*=7.5 Hz, C$\underline{H}_2$COOMe), 3.63-3.75

(4H, m, 2xCHCH$_2$OAc, CH$_3$CHO, CHOC), 3.67 (3H, s, OCH$_3$), 4.06-4.10 (2H, m, 2xCH$_a$H$_b$OAc), 4.22-4.31 (2H, m, 2xCH$_a$H$_b$OAc), 4.48 (1H, d, *J*=7.6 Hz, CH(O)$_2$), 4.73 (1H, d, *J*=8.0 Hz, CH(O)$_2$), 4.91 (1H, dxd, *J*=8.9 Hz, *J*=7.8 Hz, CHOC), 4.93 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, CHOC), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 5.29-5.40 (2H, m, CH=CH). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 20.5 (CH$_3$C=O), 20.5 (2xCH$_3$C=O), 20.6 (CH$_3$C=O), 20.7 (2xCH$_3$C=O), 20.8 (CH$_3$C=O), 21.2 (CH$_3$CH), 24.6 (CH$_2$CH$_2$COOMe), 25.1 (CH$_2$(CH$_2$)$_2$), 26.8 (CH$_2$CH=CH), 27.2 (CH$_2$CH=CH), 29.2 (CH$_2$(CH$_2$)$_2$), 29.3 (CH$_2$(CH$_2$)$_2$), 29.6 (CH$_2$(CH$_2$)$_2$), 29.7 (CH$_2$(CH$_2$)$_2$), 29.7 (2xCH$_2$(CH$_2$)$_2$), 29.8 (CH$_2$(CH$_2$)$_2$), 34.0 (CH$_2$COOMe), 36.5 (CH$_2$CHCH$_3$), 51.4 (OCH$_3$), 62.0 (CH$_2$OAc), 62.2 (CH$_2$OAc), 68.2 (CHOC), 68.9 (CHOC), 71.3 (CHOC), 71.7 (CHOC), 71.8 (CHOC), 73.1 (CHOC), 74.6 (CHOC), 77.7 (CHOC), 77.9 (CHOC), 100.4 (CH(O)$_2$), 101.1 (CH(O)$_2$), 129.0 (CH=CH), 130.5 (CH=CH), 169.3 (CH$_3$C=O), 169.4 (CH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.3 (CH$_3$C=O), 170.6 (CH$_3$C=O), 170.6 (CH$_3$C=O), 174.1 (COOMe).

General procedure for the synthesis of *(S)*-11-([2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl]-oxy)-dodecananal (212): In a 250 mL washing flask, 1.00 g peracetylated sophorolipid methyl ester (1.07 mmol, 1 eq) **211** was dissolved in 50 mL MeOH. 0.09 g $NaHCO_3$ (1.07 mmol, 1 eq) and a pinch of Sudan III indicator were added. The reaction mixture was sparged with ozone at room temperature until the red color of the reaction mixture dissipated. After addition of 0.23 g $NaBH(OAc)_3$ (1.07 mmol, 1 eq), the mixture was stirred for 1 h at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with 10 mL brine and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Peracetylated sophorolipid aldehyde **212** was purified by automated column chromatography as a viscous colorless oil with a hexane/diethyl ether mixture as eluent (3.46 g, 60%). Gradient: 2 CV 20% Et_2O, 10 CV 20-100% Et_2O, 9 CV 100% Et_2O.



212

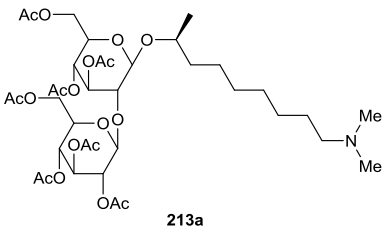
^{1}H-NMR (400 MHz, CDCl$_3$): δ_H 1.22 (3H, d, *J*=6.2 Hz, CH$_3$CH), 1.26-1.43 (13H, m, 6xCH$_2$(CH$_2$)$_2$, CH$_a$H$_b$CHCH$_3$), 1.55-1.69 (3H, CH$_a$H$_b$CHCH$_3$, CH$_2$CH$_2$(C=O)H), 1.99 (3H, s, CH$_3$C=O), 2.00 (3H, s, CH$_3$C=O), 2.01 (3H, s, CH$_3$C=O), 2.03 (3H, s, CH$_3$C=O), 2.06 (3H, s, CH$_3$C=O), 2.08 (6H, s, 2xCH$_3$C=O), 2.42 (2H, txd, *J*=7.4 Hz, *J*=1.9 Hz, CH$_2$(C=O)H), 3.63-3.74 (4H, m, 2xCHCH$_2$OAc, CH$_3$CHO, CHOC), 4.06-4.10 (2H, m, 2xCH$_a$H$_b$OAc), 4.22-4.31 (2H, m, 2xCH$_a$H$_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, CH(O)$_2$), 4.72 (1H, d, *J*=8.0 Hz, CH(O)$_2$), 4.91 (1H, dxd, *J*=9.3 Hz, *J*=8.4 Hz, CHOC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.06 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 9.76 (1H, t, *J*=1.9 Hz, HC=O). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 20.5 (CH$_3$C=O), 20.5

(2x$\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 (2x$\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.3 ($\underline{C}H_3CH$), 22.1 ($\underline{C}H_2CH_2(C=O)H$), 25.0 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.7 ($\underline{C}H_2(CH_2)_2$), 29.7 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 43.9 ($\underline{C}H_2(C=O)H$), 62.0 ($\underline{C}H_2OAc$), 62.3 ($\underline{C}H_2OAc$), 68.3 ($\underline{C}HOC$), 68.9 ($\underline{C}HOC$), 71.3 ($\underline{C}HOC$), 71.6 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 78.0 ($\underline{C}HOC$), 100.4 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 169.3 ($CH_3\underline{C}$=O), 169.4 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.3 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 203.0 ($H\underline{C}$=O).

4.2.2. Synthesis of sophorolipid amines

General procedure for the synthesis of secondary peracetylated sophororlipid amines (213): In a 50 mL flask, 1.57 g peractylated sophoroplipid aldehyde **201** (2.02 mmol, 1 eq) was dissolved in 25 mL methanol and the secondary amine (2.02 mmol, 1 eq), 0.25 g $NaBH_3CN$ (4.04 mmol, 2 eq) and 0.58 mL acetic acid (10.09 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The peracetylated sophorolipid amines were purified by automated column chromatography as a viscous colourless oil with a hexane/ethyl acetate/triethylamine mixture as eluent (mixture A = 16% triethylamine in ethyl acetate).

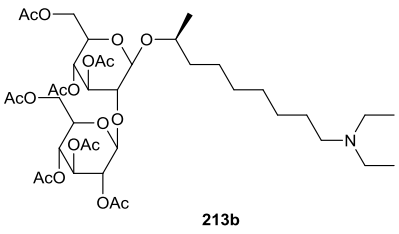
***N,N*-dimethyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-amine (213a):** Purification gradient: 2 CV 20% mixture A, 35 CV 20-60% mixture A, 2 CV 60% mixture A. (1.53 g, 38%). **IR (cm^{-1}) υ_{max}:** 1034 and 1063 (CHOCH), 1215 (COAc), 1367, 1744 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.25-1.45 (9H, m, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.52-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.68-1.76 (2H, m, $C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C$=O), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.00 (3H, s, $C\underline{H}_3C$=O), 2.03 (3H, s, 2x$C\underline{H}_3C$=O), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.08 (3H, s, $C\underline{H}_3C$=O), 2.09 (3H, s, $C\underline{H}_3C$=O), 2.70 (6H, s, 2x$C\underline{H}_3N$), 2.85-2.89 (2H, m, $C\underline{H}_2N$), 3.55-3.75 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.06-4.11 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.23-4.29 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.7 Hz, $C\underline{H}(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.87 (1H, dxd, *J*=9.6 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, $C\underline{H}OC$), 5.02 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.12 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.6 ($\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.5 ($\underline{C}H_3CH$), 24.7 ($\underline{C}H_2(CH_2)_2$), 24.8 ($\underline{C}H_2CH_2N$), 26.6 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 43.4 (2x$\underline{C}H_3N$), 58.4 ($\underline{C}H_2N$), 62.2 ($\underline{C}H_2OAc$), 62.2 ($\underline{C}H_2OAc$), 68.4 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.3 ($\underline{C}HOC$),



213a

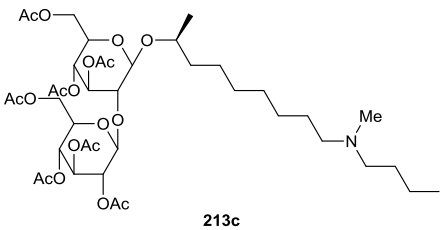
71.5 (CHOC), 71.7 (CHOC), 72.9 (CHOC), 74.8 (CHOC), 77.7 (CHOC), 78.0 (CHOC), 100.5 ($CH(O)_2$), 101.3 ($CH(O)_2$), 169.6 (2xCH_3C=O), 169.8 (CH_3C=O), 170.0 (CH_3C=O), 170.1 (CH_3C=O), 170.6 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{37}H_{60}NO_{18}$ [M+H$^+$]: 806.3805. Found: 806.3802.

***N,N*-diethyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213b):** Purification was performed *via* an acid/base extraction procedure. (0.26 g, 50%). **IR (cm^{-1}) υ_{max}:** 1034 and 1064 (CHOCH), 1218 (COAc), 1366, 1743 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 1.21 (3H, d, *J*=6.2 Hz, CH_3CH), 1.27-1.44 (9H, m, 4x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.34 (6H, t, *J*=7.3 Hz, 2xCH_3CH_2), 1.51-1.58 (1H, m, $CH_aH_bCHCH_3$), 1.68-1.76 (2H, m, CH_2CH_2N), 1.98 (3H, s, CH_3C=O), 1.99 (3H, s, CH_3C=O), 2.02 (6H, s, 2xCH_3C=O), 2.05 (3H, s, CH_3C=O), 2.07 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.94-2.98 (2H, m, CH_2CH_2N), 3.09 (4H, q, *J*=7.3 Hz, 2xCH_3CH_2N), 3.63-3.74 (4H, m, 2xCHCH$_2$OAc, CH_3CHO, CHOC), 4.04-4.10 (2H, m, 2xCHCH_aH_bOAc), 4.22-4.27 (2H, m, 2xCHCH_aH_bOAc), 4.46 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.69 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.86 (1H, dxd, *J*=9.5 Hz, *J*=8.2 Hz, CHOC), 4.92 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, CHOC), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.11 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, CHOC), 5.16 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, CHOC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 8.8 (2xCH_3CH_2), 20.4 (CH_3C=O), 20.6 (3xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.5 (CH_3CH), 23.3 (CH_2CH_2N), 24.7 ($CH_2(CH_2)_2$), 26.8 ($CH_2(CH_2)_2$), 29.2 ($CH_2(CH_2)_2$), 29.4 ($CH_2(CH_2)_2$), 36.5 (CH_2CHCH_3), 46.9 (2xCH_3CH_2N), 51.4 (CH_2CH_2N), 62.2 (2xCH_2OAc), 68.4 (CHOC), 68.8 (CHOC), 71.2 (CHOC), 71.5 (CHOC), 71.7 (CHOC), 72.9 (CHOC), 74.8 (CHOC), 77.8 (CHOC), 78.0 (CHOC), 100.5 ($CH(O)_2$), 101.3 ($CH(O)_2$), 169.6 (2xCH_3C=O), 169.7 (CH_3C=O), 170.0 (CH_3C=O), 170.1 (CH_3C=O), 170.6 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{39}H_{64}NO_{18}$ [M+H$^+$]: 834.4118. Found: 834.4111.



213b

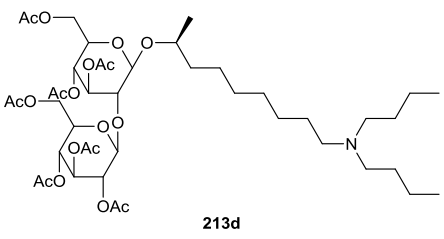
***N*-butyl,*N*-methyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213c):** Purification gradient: 2 CV 15% mixture A, 35 CV 15-55% mixture A, 2 CV 55% mixture A. (1.12 g, 52%). **IR (cm^{-1}) υ_{max}:** 1033 and 1063 (CHOCH), 1216 (COAc), 1366, 1745 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 0.95 (3H, t, *J*=7.3 Hz, CH_3CH_2), 1.22 (3H, d, *J*=6.2 Hz, CH_3CH), 1.26-1.42 (11H, m, 5x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.54-1.66 (5H, m, 2xCH_2CH_2N, $CH_aH_bCHCH_3$), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.02 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.48 (3H, s, CH_3N), 2.63-2.68 (4H, m, 2xCH_2N), 3.64-3.76 (4H, m, 2xCHCH$_2$OAc, CH_3CHO, CHOC), 4.06-4.11 (2H, m, 2xCHCH_aH_bOAc), 4.22-4.29 (2H, m, 2xCHCH_aH_bOAc), 4.47 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.72 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.89 (1H,



213c

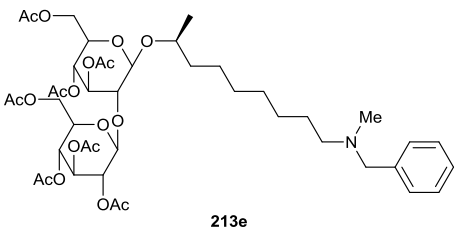
dxd, *J*=9.5 Hz, *J*=8.1 Hz, CHOC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.03 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 13.8 (CH_3CH_2), 20.4 (CH_3CH_2), 20.5 (CH_3C=O), 20.6 (3xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.4 (CH_3CH), 24.8 (CH_2CH_2N), 25.4 ($CH_2(CH_2)_2$), 27.2 ($CH_2(CH_2)_2$), 27.6 (CH_2CH_2N), 29.5 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 36.5 (CH_2CHCH_3), 41.0 (CH_3N), 56.6 (CH_2N), 56.9 (CH_2N), 62.1 (CH_2OAc), 62.2 (CH_2OAc), 68.3 (CHOC), 68.8 (CHOC), 71.3 (CHOC), 71.6 (CHOC), 71.8 (CHOC), 73.0 (CHOC), 74.7 (CHOC), 77.7 (CHOC), 77.9 (CHOC), 100.4 ($CH(O)_2$), 101.2 ($CH(O)_2$), 169.4 (CH_3C=O), 169.5 (CH_3C=O), 169.7 (CH_3C=O), 170.0 (CH_3C=O), 170.2 (CH_3C=O), 170.6 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{40}H_{66}NO_{18}$ [M+H$^+$]: 848.4274. Found: 848.4305.

***N,N*-dibutyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213d):** Purification gradient: 2 CV 5% mixture A, 35 CV 5-50% mixture A, 2 CV 50% mixture A. (0.71 g, 53%). **IR (cm^{-1}) υ_{max}:** 1034 and 1064 (CHOCH), 1217 (COAc), 1366, 1745 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.97 (6H, t, *J*=7.3 Hz, 2xCH_3CH_2), 1.22 (3H, d, *J*=6.2 Hz, CH_3CH), 1.26-1.43 (13H, m, $CH_aH_bCHCH_3$, 2xCH_2CH_3, 4x$CH_2(CH_2)_2$), 1.54-1.60 (1H, m, $CH_aH_bCHCH_3$), 1.61-1.72 (6H, m, 3xCH_2CH_2N), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.02 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.09 (3H, s, CH_3C=O), 2.83-2.88 (6H, m, 3xCH_2N), 3.64-3.75 (4H, m, 2xCHCH$_2$OAc, CH_3CHO, CHOC), 4.06-4.10 (2H, m, 2xCHCH_aH_bOAc), 4.23-4.29 (2H, m, 2xCHCH_aH_bOAc), 4.47 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.88 (1H, dxd, *J*=9.4 Hz, *J*=8.0 Hz, CHOC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.03 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.13 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 13.6 (2xCH_3CH_2), 20.2 (2xCH_3CH_2), 20.4 (CH_3C=O), 20.5 (3xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.4 (CH_3CH), 24.0 (CH_2CH_2N), 24.8 ($CH_2(CH_2)_2$), 26.2 (2xCH_2CH_2N), 27.0 ($CH_2(CH_2)_2$), 29.4 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 36.5 (CH_2CHCH_3), 52.7 (2xCH_2N), 52.9 (CH_2N), 62.1 (CH_2OAc), 62.2 (CH_2OAc), 68.4 (CHOC), 68.8 (CHOC), 71.2 (CHOC), 71.5 (CHOC), 71.7 (CHOC), 72.9 (CHOC), 74.7 (CHOC), 77.6 (CHOC), 77.9 (CHOC), 100.4 ($CH(O)_2$), 101.2 ($CH(O)_2$), 169.4 (CH_3C=O), 169.5 (CH_3C=O), 169.7 (CH_3C=O), 170.0 (CH_3C=O), 170.1 (CH_3C=O), 170.5 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{43}H_{72}NO_{18}$ [M+H$^+$]: 890.4744. Found: 890.4761.



213d

***N*-methyl,*N*-benzyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213e):** Purification gradient: 2 CV 5% mixture A, 35 CV 5-50% mixture A, 2 CV 50% mixture A. (1.19 g, 40%). **IR (cm^{-1}) υ_{max}:** 733, 1033 and 1063 (CHOCH), 1214 (COAc), 1366, 1746 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (3H, t, *J*=6.1 Hz, C<u>H</u>$_3$CH), 1.25-1.42 (9H, m, 4xC<u>H</u>$_2$(CH$_2$)$_2$, C<u>H</u>$_a$H$_b$CHCH$_3$), 1.51-1.63 (3H, m, C<u>H</u>$_2$CH$_2$N, CH$_a$<u>H</u>$_b$CHCH$_3$), 1.98 (3H, s, C<u>H</u>$_3$C=O), 2.00 (3H, s, C<u>H</u>$_3$C=O), 2.01 (3H, s, C<u>H</u>$_3$C=O), 2.03 (3H, s, C<u>H</u>$_3$C=O), 2.06 (3H, s, C<u>H</u>$_3$C=O), 2.07 (3H, s, C<u>H</u>$_3$C=O), 2.08 (3H, s, C<u>H</u>$_3$C=O), 2.18 (3H, s, C<u>H</u>$_3$N), 2.38 (2H, t, *J*=7.5 Hz, C<u>H</u>$_2$N), 3.48 (2H, s, C$_{arom}$C<u>H</u>$_2$N), 3.63-3.75 (4H, m, 2xC<u>H</u>CH$_2$OAc, CH$_3$C<u>H</u>O, C<u>H</u>OC), 4.06-4.10 (2H, m, 2xCHC<u>H</u>$_a$H$_b$OAc), 4.22-4.31 (2H, m, 2xCHCH$_a$<u>H</u>$_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C<u>H</u>(O)$_2$), 4.73 (1H, d, *J*=8.0 Hz, C<u>H</u>(O)$_2$), 4.91 (1H, dxd, *J*=9.2 Hz, *J*=7.8 Hz, C<u>H</u>OC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C<u>H</u>OC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C<u>H</u>OC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, C<u>H</u>OC), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C<u>H</u>OC), 7.21-7.31 (5H, m, 5xC<u>H</u>$_{arom}$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (<u>C</u>H$_3$C=O), 20.6 (2x<u>C</u>H$_3$C=O), 20.6 (<u>C</u>H$_3$C=O), 20.7 (2x<u>C</u>H$_3$C=O), 20.8 (<u>C</u>H$_3$C=O), 21.2 (<u>C</u>H$_3$CH), 25.0 (<u>C</u>H$_2$(CH$_2$)$_2$), 27.4 (<u>C</u>H$_2$CH$_2$N), 27.5 (<u>C</u>H$_2$(CH$_2$)$_2$), 29.7 (<u>C</u>H$_2$(CH$_2$)$_2$), 29.8 (<u>C</u>H$_2$(CH$_2$)$_2$), 36.4 (<u>C</u>H$_2$CHCH$_3$), 42.2 (<u>C</u>H$_3$N), 57.6 (CH$_2$<u>C</u>H$_2$N), 62.0 (C$_{arom}$<u>C</u>H$_2$N, <u>C</u>H$_2$OAc), 62.3 (<u>C</u>H$_2$OAc), 68.3 (<u>C</u>HOC), 68.9 (<u>C</u>HOC), 71.3 (<u>C</u>HOC), 71.7 (<u>C</u>HOC), 71.8 (<u>C</u>HOC), 73.1 (<u>C</u>HOC), 74.6 (<u>C</u>HOC), 77.7 (<u>C</u>HOC), 77.9 (<u>C</u>HOC), 100.4 (<u>C</u>H(O)$_2$), 101.1 (<u>C</u>H(O)$_2$), 126.8 (<u>C</u>H$_{arom}$), 128.1 (2x<u>C</u>H$_{arom}$), 129.0 (2x<u>C</u>H$_{arom}$), 139.3 (<u>C</u>$_{arom}$), 169.3 (CH$_3$<u>C</u>=O), 169.4 (CH$_3$<u>C</u>=O), 169.7 (CH$_3$<u>C</u>=O), 170.0 (CH$_3$<u>C</u>=O), 170.3 (CH$_3$<u>C</u>=O), 170.6 (CH$_3$<u>C</u>=O), 170.6 (CH$_3$<u>C</u>=O). **MS (ESI): m/z** Exact mass calculated for $C_{43}H_{64}NO_{18}$ [M+H$^+$]: 882.4118. Found: 882.4160.



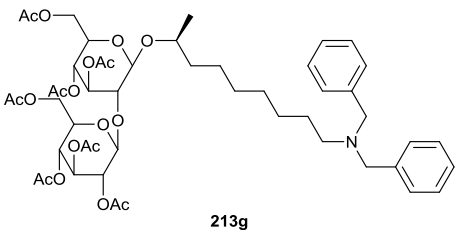
213e

***N*-butyl,*N*-benzyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213f):** Purification gradient: 2 CV 5% mixture A, 35 CV 5-50% mixture A, 2 CV 50% mixture A. (1.92 g, 49%). **IR (cm^{-1}) υ_{max}:** 1034 and 1064 (CHOCH), 1213 (COAc), 1365, 1747 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.87 (3H, t, *J*=7.3 Hz, C<u>H</u>$_3$CH$_2$), 1.21 (3H, d, *J*=6.2 Hz, C<u>H</u>$_3$CH), 1.24-1.39 (11H, m, C<u>H</u>$_2$CH$_3$, 4xC<u>H</u>$_2$(CH$_2$)$_2$, C<u>H</u>$_a$H$_b$CHCH$_3$), 1.40-1.50 (4H, m, 2x<u>C</u>H$_2$CH$_2$N), 1.56-1.62 (1H, m, CH$_a$<u>H</u>$_b$CHCH$_3$), 1.97 (3H, s, C<u>H</u>$_3$C=O), 2.00 (3H, s, C<u>H</u>$_3$C=O), 2.00 (3H, s, C<u>H</u>$_3$C=O), 2.03 (3H, s, C<u>H</u>$_3$C=O), 2.06 (3H, s, C<u>H</u>$_3$C=O), 2.07 (3H, s, C<u>H</u>$_3$C=O), 2.08 (3H, s, C<u>H</u>$_3$C=O), 2.38-2.42 (4H, m, 2xCH$_2$C<u>H</u>$_2$N), 3.54 (2H, s, C$_{arom}$C<u>H</u>$_2$N), 3.62-3.74 (4H, m, 2xC<u>H</u>CH$_2$OAc, CH$_3$C<u>H</u>O, C<u>H</u>OC), 4.06-4.10 (2H, m, 2xCHC<u>H</u>$_a$H$_b$OAc), 4.22-4.31 (2H, m, 2xCHCH$_a$<u>H</u>$_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C<u>H</u>(O)$_2$), 4.73 (1H, d, *J*=8.0 Hz, C<u>H</u>(O)$_2$), 4.87-4.95 (2H, m, 2xC<u>H</u>OC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C<u>H</u>OC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, C<u>H</u>OC), 5.16 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, C<u>H</u>OC), 7.19-7.23 (1H, m, C<u>H</u>$_{arom}$), 7.27-7.34

213f

(4H, m, C$\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 14.1 (C$\underline{H}_3$CH$_2$), 20.5 (C$\underline{H}_3$C=O), 20.5 (2xC$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (CH$_3$C$\underline{H}_2$), 20.7 (2xC$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 21.2 (C$\underline{H}_3$CH), 25.1 (C$\underline{H}_2$(CH$_2$)$_2$), 27.0 (C$\underline{H}_2$CH$_2$N), 27.5 (C$\underline{H}_2$(CH$_2$)$_2$), 29.2 (C$\underline{H}_2$CH$_2$N), 29.7 (2xC$\underline{H}_2$(CH$_2$)$_2$), 36.4 (C$\underline{H}_2$CHCH$_3$), 53.5 (CH$_2$C$\underline{H}_2$N), 53.8 (CH$_2$C$\underline{H}_2$N), 58.6 (C$_{arom}$C$\underline{H}_2$N), 62.0 (C$\underline{H}_2$OAc), 62.3 (C$\underline{H}_2$OAc), 68.2 (C$\underline{H}$OC), 68.9 (C$\underline{H}$OC), 71.3 (C$\underline{H}$OC), 71.7 (C$\underline{H}$OC), 71.8 (C$\underline{H}$OC), 73.0 (C$\underline{H}$OC), 74.6 (C$\underline{H}$OC), 77.7 (C$\underline{H}$OC), 78.0 (C$\underline{H}$OC), 100.4 (C$\underline{H}$(O)$_2$), 101.1 (C$\underline{H}$(O)$_2$), 126.5 (C$\underline{H}_{arom}$), 128.0 (2xC$\underline{H}_{arom}$), 128.8 (2xC$\underline{H}_{arom}$), 140.3 (C$_{arom}$), 169.3 (CH$_3$C=O), 169.4 (CH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.3 (CH$_3$C=O), 170.6 (CH$_3$C=O), 170.6 (CH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{46}H_{70}NO_{18}$ [M+H$^+$]: 924.4587. Found: 924.4625.

***N,N*-dibenzyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (213g):** Purification gradient: 2 CV 1% mixture A, 35 CV 1-50% mixture A, 2 CV 50% mixture A. (1.33 g, 48%). **IR (cm^{-1}) υ_{max}:** 733, 1033 and 1064 (CHOCH), 1214 (COAc), 1366, 1746 (C=O). **^{1}H-NMR (400 MHz, CDCl$_3$): δ_H** 1.21 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.22-1.39 (9H, m, 4xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.50-1.61 (3H, m, C$\underline{H}_2$CH$_2$N, CH$_a$H$_b$CHCH$_3$), 1.96 (3H, s, C$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.02 (3H, s, C$\underline{H}_3$C=O), 2.05 (3H, s, C$\underline{H}_3$C=O), 2.05 (3H, s, C$\underline{H}_3$C=O), 2.07 (3H, s, C$\underline{H}_3$C=O), 2.41 (2H, t, *J*=7.2 Hz, CH$_2$C$\underline{H}_2$N), 3.55 (4H, s, 2xC$_{arom}$C$\underline{H}_2$N), 3.62-3.74 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.06-4.10 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.30 (2H, m, 2xCHCH$_a$$\underline{H}_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.73 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.91 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, C$\underline{H}$OC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, C$\underline{H}$OC), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 7.19-7.37 (10H, m, 10xC$\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 20.5 (C$\underline{H}_3$C=O), 20.5 (2xC$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.7 (2xC$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 21.2 (C$\underline{H}_3$CH), 25.0 (C$\underline{H}_2$(CH$_2$)$_2$), 26.9 (C$\underline{H}_2$CH$_2$N), 27.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.7 (C$\underline{H}_2$(CH$_2$)$_2$), 29.7 (C$\underline{H}_2$(CH$_2$)$_2$), 36.4 (C$\underline{H}_2$CHCH$_3$), 53.4 (CH$_2$C$\underline{H}_2$N), 58.2 (2xC$_{arom}$C$\underline{H}_2$N), 62.0 (C$\underline{H}_2$OAc), 62.3 (C$\underline{H}_2$OAc), 68.2 (C$\underline{H}$OC), 68.9 (C$\underline{H}$OC), 71.3 (C$\underline{H}$OC), 71.7 (C$\underline{H}$OC), 71.8 (C$\underline{H}$OC), 73.1 (C$\underline{H}$OC), 74.6 (C$\underline{H}$OC), 77.7 (C$\underline{H}$OC), 77.9 (C$\underline{H}$OC), 100.4 (C$\underline{H}$(O)$_2$), 101.1 (C$\underline{H}$(O)$_2$), 126.7 (2xC$\underline{H}_{arom}$), 128.1 (4xC$\underline{H}_{arom}$), 128.8 (4xC$\underline{H}_{arom}$), 140.0 (2xC$_{arom}$), 169.3 (CH$_3$C=O), 169.4 (CH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.3 (CH$_3$C=O), 170.6 (CH$_3$C=O), 170.6 (CH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{49}H_{68}NO_{18}$ [M+H$^+$]: 958.4431. Found: 958.4421.



213g

***N*-methyl,*N*-octadecyl-((*S*)-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-amine (213h):** Purification gradient: 2 CV 10% mixture A, 35 CV 10-50% mixture A, 2 CV 50% mixture A. (2.11 g, 39%).


213h

IR (cm^{-1}) υ_{max}: 1034 and 1063 (CHOCH), 1216 (COAc), 1366, 1747 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 0.88 (3H, t, *J*=6.8 Hz, C$\underline{H}_3$CH$_2$), 1.21 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.25-1.40 (39H, m, C$\underline{H}_2$CH$_3$, 18xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.41-1.51 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 1.56-1.62 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.99 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.01 (3H, s, C$\underline{H}_3$C=O), 2.03 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.20 (3H, s, C$\underline{H}_3$N), 2.28-2.33 (4H, m, 2xCH$_2$C$\underline{H}_2$N), 3.63-3.75 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.06-4.10 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.31 (2H, m, 2xCHCH$_a\underline{H}_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.73 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.89-4.95 (2H, m, 2xC$\underline{H}$OC), 5.06 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, C$\underline{H}$OC), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 14.1 (C$\underline{H}_3$CH$_2$), 20.5 (C$\underline{H}_3$C=O), 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.7 (2xC$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 21.2 (C$\underline{H}_3$CH), 22.7 (CH$_3$C$\underline{H}_2$), 25.0 (C$\underline{H}_2$(CH$_2$)$_2$), 27.3 (C$\underline{H}_2$CH$_2$N), 27.4 (C$\underline{H}_2$CH$_2$N), 27.7 (C$\underline{H}_2$(CH$_2$)$_2$), 27.7 (C$\underline{H}_2$(CH$_2$)$_2$), 29.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.6-29.7 (12xC$\underline{H}_2$(CH$_2$)$_2$), 29.8 (C$\underline{H}_2$(CH$_2$)$_2$), 31.9 (C$\underline{H}_2$(CH$_2$)$_2$), 36.5 (C$\underline{H}_2$CHCH$_3$), 42.3 (C$\underline{H}_3$N), 58.0 (CH$_2$C$\underline{H}_2$N), 58.0 (CH$_2$C$\underline{H}_2$N), 62.0 (C$\underline{H}_2$OAc), 62.3 (C$\underline{H}_2$OAc), 68.2 (C$\underline{H}$OC), 68.9 (C$\underline{H}$OC), 71.3 (C$\underline{H}$OC), 71.7 (C$\underline{H}$OC), 71.8 (C$\underline{H}$OC), 73.1 (C$\underline{H}$OC), 74.6 (C$\underline{H}$OC), 77.7 (C$\underline{H}$OC), 77.9 (C$\underline{H}$OC), 100.4 (C$\underline{H}$(O)$_2$), 101.1 (C$\underline{H}$(O)$_2$), 169.3 (CH$_3\underline{C}$=O), 169.4 (CH$_3\underline{C}$=O), 169.7 (CH$_3\underline{C}$=O), 170.0 (CH$_3\underline{C}$=O), 170.3 (CH$_3\underline{C}$=O), 170.6 (CH$_3\underline{C}$=O), 170.6 (CH$_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{54}H_{94}NO_{18}$ [M+H$^+$]: 1044.6465. Found: 1044.6499.

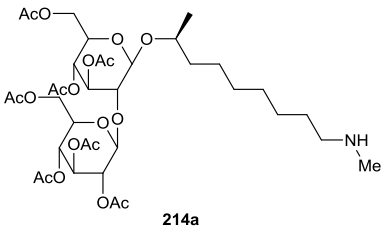
***N*-methyl,*N*-octadecyl-((*S*)-11-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])dodecan-1-amine (226):** Purification gradient: 2 CV 15% mixture A, 25 CV 15-60% mixture A, 2 CV 60% mixture A. (1.22 g, 46%).


226

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 0.88 (3H, t, *J*=6.8 Hz, C$\underline{H}_3$CH$_2$), 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.24-1.40 (45H, m, C$\underline{H}_2$CH$_3$, 21xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.41-1.50 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 1.56-1.66 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.99 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.01 (3H, s, C$\underline{H}_3$C=O), 2.03 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.20 (3H, s, C$\underline{H}_3$N), 2.28-2.32 (4H, m, 2xCH$_2$C$\underline{H}_2$N), 3.63-3.75 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.06-4.10 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.23-4.32 (2H, m, 2xCHCH$_a\underline{H}_b$OAc), 4.48 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.74 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.89-4.96 (2H, m, 2xC$\underline{H}$OC), 5.07 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 5.13 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, C$\underline{H}$OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 14.1 (C$\underline{H}_3$CH$_2$), 20.5 (C$\underline{H}_3$C=O), 20.6 (2xC$\underline{H}_3$C=O), 20.6

(CH_3C=O), 20.7 (2xCH_3C=O), 20.8 (CH_3C=O), 21.2 (CH_3CH), 22.7 (CH$_3$CH_2), 25.1 (CH_2(CH$_2$)$_2$), 27.3 (CH_2CH$_2$N), 27.4 (CH_2CH$_2$N), 27.6 (CH_2(CH$_2$)$_2$), 27.7 (CH_2(CH$_2$)$_2$), 29.3 (CH_2(CH$_2$)$_2$), 29.6-29.7 (14xCH_2(CH$_2$)$_2$), 29.7 (CH_2(CH$_2$)$_2$), 29.8 (CH_2(CH$_2$)$_2$), 31.9 (CH_2(CH$_2$)$_2$), 36.5 (CH_2CHCH$_3$), 42.3 (CH_3N), 58.0 (CH$_2$CH_2N), 58.0 (CH$_2$CH_2N), 61.9 (CH_2OAc), 62.2 (CH_2OAc), 68.2 (CHOC), 68.8 (CHOC), 71.3 (CHOC), 71.7 (CHOC), 71.8 (CHOC), 73.0 (CHOC), 74.6 (CHOC), 77.6 (CHOC), 78.0 (CHOC), 100.4 (CH(O)$_2$), 101.1 (CH(O)$_2$), 169.3 (CH$_3$C=O), 169.4 (CH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.3 (CH$_3$C=O), 170.6 (CH$_3$C=O), 170.6 (CH$_3$C=O).

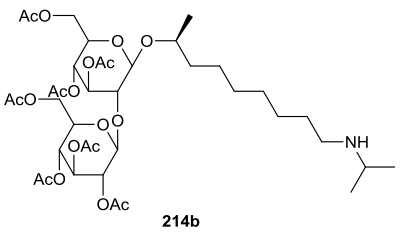
General procedure for the synthesis of *N*-methyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (214a): In a 50 mL flask, 0.51 g peractylated sophoroplipid aldehyde **201** (3.25 mmol, 5 eq) was dissolved in 25 mL methanol and after addition of 1.63 ml of methyl amine (0.65 mmol, 1 eq), the mixture was stirred at room temperature. After 1 hour, 0.08 g $NaBH_3CN$ (1.30 mmol, 2 eq) and 0.19 mL acetic acid (3.25 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Methyl sophorolipid amine **214a** was obtained in high purity without further purification (0.31 g, 60%).



214a

^{1}H-NMR (400 MHz, CDCl$_3$): δ_H 1.22 (3H, d, *J*=6.2 Hz, CH_3CH), 1.26-1.43 (9H, m, 4xCH_2(CH$_2$)$_2$, CH_aH$_b$CHCH$_3$), 1.54-1.66 (3H, m, CH$_a$H$_b$CHCH$_3$, CH_2CH$_2$N), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.08 (6H, s, 2xCH_3C=O), 2.55 (3H, s, CH_3N), 2.76 (2H, t, *J*=7.5 Hz, CH_2N), 3.64-3.75 (4H, m, 2xCHCH$_2$OAc, CH$_3$CHO, CHOC), 3.96 (1H, br s, NH), 4.07-4.10 (2H, m, 2xCHCH_aH$_b$OAc), 4.22-4.30 (2H, m, 2xCHCH$_a$H$_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, CH(O)$_2$), 4.72 (1H, d, *J*=8.0 Hz, CH(O)$_2$), 4.89 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, CHOC), 4.93 (1H, dxd, J=9.7 Hz, J=9.7 Hz, CHOC), 5.04 (1H, dxd, J=9.6 Hz, J=9.6 Hz, CHOC), 5.13 (1H, dxd, J=9.4 Hz, J=9.4 Hz, CHOC), 5.17 (1H, dxd, J=9.5 Hz, J=9.5 Hz, CHOC). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 20.5 (CH_3C=O), 20.6 (3xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.4 (CH_3CH), 24.8 (CH_2(CH$_2$)$_2$), 26.9 (CH_2(CH$_2$)$_2$), 28.0 (CH_2CH$_2$N), 29.5 (CH_2(CH$_2$)$_2$), 29.5 (CH_2(CH$_2$)$_2$), 34.9 (CH_3N), 36.5 (CH_2CHCH$_3$), 50.9 (CH_2N), 62.1 (CH_2OAc), 62.3 (CH_2OAc), 68.4 (CHOC), 68.8 (CHOC), 71.3 (CHOC), 71.6 (CHOC), 71.8 (CHOC), 73.0 (CHOC), 74.7 (CHOC), 77.6 (CHOC), 78.0 (CHOC), 100.4 (CH(O)$_2$), 101.2 (CH(O)$_2$), 169.6 (CH$_3$C=O), 169.7 (CH$_3$C=O), 169.8 (CH$_3$C=O), 170.0 (CH$_3$C=O), 170.2 (CH$_3$C=O), 170.6 (CH$_3$C=O), 170.7 (CH$_3$C=O).

General procedure for the synthesis of *N*-isopropyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine (214b): In a 50 mL flask, 0.16 g peractylated sophoroplipid aldehyde **201** (0.21 mmol, 1 eq) was dissolved in 25 mL methanol and 0.72 ml isopropyl amine (0.42 mmol, 2 eq), 0.03 g $NaBH_3CN$ (0.42 mmol, 2 eq) and 0.06 mL acetic acid (1.06 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Methyl sophorolipid amine **214b** was obtained in high purity without further purification (0.13 g, 73%).



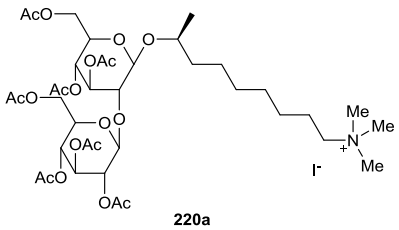
214b

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 1.22 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CHCH_2$), 1.24-1.47 (9H, m, $4xC\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.42 (6H, d, *J*=6.5 Hz, 2x $C\underline{H}_3CHN$), 1.54-1.63 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.76-1.84 (2H, m, $\underline{C}H_2CH_2N$), 2.00 (6H, s, $2xC\underline{H}_3C=O$), 2.04 (6H, s, $2xC\underline{H}_3C=O$), 2.06 (3H, s, $C\underline{H}_3C=O$), 2.09 (3H, s, $C\underline{H}_3C=O$), 2.09 (3H, s, $C\underline{H}_3C=O$), 2.98 (2H, t, *J*=7.9 Hz, $C\underline{H}_2N$), 3.37 (1H, sept, *J*=6.5 Hz, $C\underline{H}N$), 3.65-3.76 (4H, m, $2xC\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.07-4.11 (2H, m, $2xCHC\underline{H}_aH_bOAc$), 4.23-4.29 (2H, m, $2xCHCH_a\underline{H}_bOAc$), 4.50 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.73 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.86-4.90 (1H, m, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.03 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$), 5.18 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 18.8 (2x $C\underline{H}_3CHN$), 20.5 ($\underline{C}H_3C=O$), 20.6 ($2x\underline{C}H_3C=O$), 20.6 ($\underline{C}H_3C=O$), 20.7 ($\underline{C}H_3C=O$), 20.8 ($2x\underline{C}H_3C=O$), 21.4 ($\underline{C}H_3CHCH_2$), 24.7 ($\underline{C}H_2(CH_2)_2$), 25.9 ($\underline{C}H_2(CH_2)_2$), 26.5 ($\underline{C}H_2CH_2N$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 45.2 ($\underline{C}H_2N$), 51.0 ($\underline{C}HN$), 62.2 ($2x\underline{C}H_2OAc$), 68.4 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.2 ($\underline{C}HOC$), 71.6 ($\underline{C}HOC$), 71.7 ($\underline{C}HOC$), 72.9 ($\underline{C}HOC$), 74.6 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 77.9 ($\underline{C}HOC$), 100.3 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 169.7 ($2xCH_3\underline{C}=O$), 169.8 ($CH_3\underline{C}=O$), 170.0 ($CH_3\underline{C}=O$), 170.2 ($CH_3\underline{C}=O$), 170.7 ($CH_3\underline{C}=O$), 170.8 ($CH_3\underline{C}=O$).

4.2.3. Synthesis of quaternary ammonium sophorolipids

General procedure for synthesis of peracetylated sophorolipid quaternary ammonium salts: In a 10 mL flame dried pressure resistant vial, peracetylated sophorolipid amine was dissolved in dry acetonitrile. The solution was cooled down to 0 °C and the alkyl iodide (5 eq) was added. The vial was closed and heated to 80 °C for the specified reaction time. The reaction mixture was concentrated under reduced pressure and recrystallized from diethyl ether if necessary to yield the peracetylated sophorolipid quaternary ammonium salt.

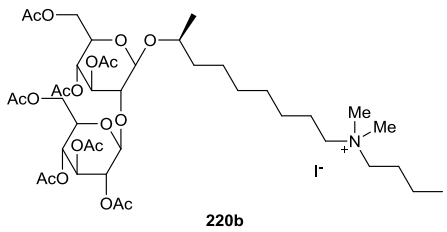
***N*,*N*,*N*-trimethyl-((*S*)-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220a):** (0.82 g, 91%), orange-yellow powder,



220a

mp 85 °C. **IR (cm^{-1}) υ_{max}:** 729, 917, 1033 (CHOCH), 1216 (COAc), 1366, 1741 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ 1.22 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.25-1.47 (9H, m, $4xC\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.51-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.80-1.88 (2H, m, $C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C=O$), 1.99 (3H, s, $C\underline{H}_3C=O$), 2.02 (3H, s, $C\underline{H}_3C=O$), 2.03 (3H, s, $C\underline{H}_3C=O$), 2.06 (3H, s, $C\underline{H}_3C=O$), 2.08 (3H, s, $C\underline{H}_3C=O$), 2.09 (3H, s, $C\underline{H}_3C=O$), 3.47 (9H, s, $3xC\underline{H}_3N$), 3.54-3.58 (2H, m, $CH_2C\underline{H}_2N$), 3.65-3.74 (4H, m, $2xC\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.05-4.11 (2H, m, $2xCHC\underline{H}_aH_bOAc$), 4.22-4.27 (2H, m, $2xCHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.70 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.86 (1H, dxd, *J*=9.6 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 4.92 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 4.99 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.11 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ 20.5 ($C\underline{H}_3C=O$), 20.6 ($C\underline{H}_3C=O$), 20.7 ($C\underline{H}_3C=O$), 20.7 ($2xC\underline{H}_3C=O$), 20.8 ($2xC\underline{H}_3C=O$), 21.6 ($C\underline{H}_3CH$), 23.0 ($C\underline{H}_2CH_2N$), 24.6 ($C\underline{H}_2(CH_2)_2$), 26.1 ($C\underline{H}_2(CH_2)_2$), 29.3 ($C\underline{H}_2(CH_2)_2$), 29.4 ($C\underline{H}_2(CH_2)_2$), 36.6 ($C\underline{H}_2CHCH_3$), 53.6 ($3xC\underline{H}_3N$), 62.2 ($C\underline{H}_2OAc$), 62.4 ($C\underline{H}_2OAc$), 67.4 ($CH_2C\underline{H}_2N$), 68.5 ($C\underline{H}OC$), 68.9 ($C\underline{H}OC$), 71.2 ($C\underline{H}OC$), 71.4 ($C\underline{H}OC$), 71.7 ($C\underline{H}OC$), 72.9 ($C\underline{H}OC$), 74.8 ($C\underline{H}OC$), 77.8 ($C\underline{H}OC$), 78.0 ($C\underline{H}OC$), 100.5 ($C\underline{H}(O)_2$), 101.3 ($C\underline{H}(O)_2$), 169.7 ($2xCH_3\underline{C}=O$), 169.7 ($CH_3\underline{C}=O$), 170.0 ($CH_3\underline{C}=O$), 170.0 ($CH_3\underline{C}=O$), 170.6 ($CH_3\underline{C}=O$), 170.6 ($CH_3\underline{C}=O$). **MS (ESI): m/z** Exact mass calculated for $C_{38}H_{62}NO_{18}$ [M-I$^-$]: 820.3961. Found: 820.3963.

***N*-butyl,*N*,*N*-dimethyl-((*S*)-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220b):** (0.44 g, 89%), orange-yellow powder, **mp**

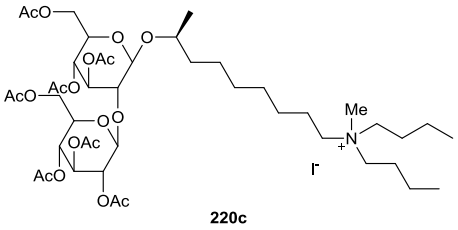


220b

73 °C. **IR (cm^{-1}) υ_{max}:** 728, 918, 1034 (CHOCH), 1218 (COAc), 1366, 1745 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ 1.02 (3H, t, *J*=7.3 Hz, $CH_2C\underline{H}_3$), 1.22 (3H, d, *J*=6.1 Hz, $CHC\underline{H}_3$), 1.26-1.50 (11H, m, $C\underline{H}_2CH_3$, $4xC\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.52-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.71-1.84 (4H, m, $2xC\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C=O$), 2.00 (3H, s, $C\underline{H}_3C=O$), 2.03 (6H, s, $2xC\underline{H}_3C=O$), 2.06 (3H, s, $C\underline{H}_3C=O$), 2.08 (3H, s, $C\underline{H}_3C=O$), 2.09 (3H, s, $C\underline{H}_3C=O$), 3.39 (6H, s, $2xC\underline{H}_3N$), 3.45-3.50 (2H, m, $CH_2C\underline{H}_2N$), 3.59-3.63 (2H, m, $CH_2C\underline{H}_2N$), 3.66-3.74 (4H, m, $2xC\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.05-4.11 (2H, m, $2xCHC\underline{H}_aH_bOAc$), 4.22-4.27 (2H, m, $2xCHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.70 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.84-4.89 (1H, m, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 4.99 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.11 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ 13.8 ($CH_2C\underline{H}_3$), 19.6 ($C\underline{H}_2CH_3$), 20.5 ($C\underline{H}_3C=O$), 20.6 ($C\underline{H}_3C=O$), 20.7 ($2xC\underline{H}_3C=O$), 20.7 ($C\underline{H}_3C=O$), 20.8 ($C\underline{H}_3C=O$), 20.8 ($C\underline{H}_3C=O$), 21.6 ($C\underline{H}_3CH$), 22.7 ($C\underline{H}_2CH_2N$), 24.7 ($C\underline{H}_2CH_2N$, $C\underline{H}_2(CH_2)_2$), 26.3 ($C\underline{H}_2(CH_2)_2$),

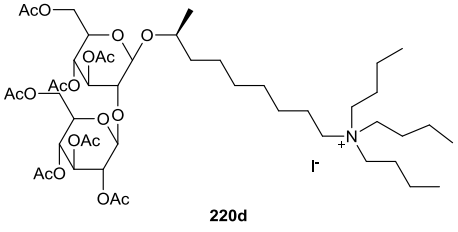
29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 36.6 ($\underline{C}H_2CHCH_3$), 51.5 (2x$\underline{C}H_3N$), 62.2 ($\underline{C}H_2OAc$), 62.4 ($\underline{C}H_2OAc$), 64.1 ($CH_2\underline{C}H_2N$), 64.3 ($CH_2\underline{C}H_2N$), 68.6 ($\underline{C}HOC$), 68.9 ($\underline{C}HOC$), 71.3 ($\underline{C}HOC$), 71.4 ($\underline{C}HOC$), 71.7 ($\underline{C}HOC$), 72.9 ($\underline{C}HOC$), 74.8 ($\underline{C}HOC$), 77.8 ($\underline{C}HOC$), 78.0 ($\underline{C}HOC$), 100.5 ($\underline{C}H(O)_2$), 101.3 ($\underline{C}H(O)_2$), 169.7 (2x$CH_3\underline{C}$=O), 169.8 ($CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{41}H_{68}NO_{18}$ [M-I$^-$]: 862.4431. Found: 862.4443.

***N*,*N*-dibutyl,*N*-methyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220c):** (0.80 g, 96%), yellow powder, **mp** 70 °C. **IR (cm^{-1}) υ_{max}:** 728, 917, 1032 and 1062 (CHOCH), 1216 (COAc), 1366, 1742 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 1.02 (6H, t, *J*=7.3 Hz, 2x$CH_2C\underline{H}_3$), 1.22 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.26-1.52 (13H, m, 2x$C\underline{H}_2CH_3$, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.54-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.70-1.79 (6H, m, 3x$C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.00 (3H, s, $C\underline{H}_3C$=O), 2.03 (6H, s, 2x$C\underline{H}_3C$=O), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.08 (3H, s, $C\underline{H}_3C$=O), 2.09 (3H, s, $C\underline{H}_3C$=O), 3.31 (3H, s, $C\underline{H}_3N$), 3.38-3.43 (2H, m, $CH_2C\underline{H}_2N$), 3.45-3.57 (4H, m, 2x$CH_2C\underline{H}_2N$), 3.65-3.75 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.05-4.11 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.22-4.27 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.87 (1H, dxd, *J*=9.6 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.12 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 13.7 (2x$CH_2\underline{C}H_3$), 19.7 (2x$\underline{C}H_2CH_3$), 20.5 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.5 ($\underline{C}H_3CH$), 22.4 ($\underline{C}H_2CH_2N$), 24.4 (2x$\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.4 ($\underline{C}H_2(CH_2)_2$), 29.4 (2x$\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 49.1 ($\underline{C}H_3N$), 61.6 ($CH_2\underline{C}H_2N$), 61.7 (2x$CH_2\underline{C}H_2N$), 62.2 ($\underline{C}H_2OAc$), 62.3 ($\underline{C}H_2OAc$), 68.5 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.2 ($\underline{C}HOC$), 71.4 ($\underline{C}HOC$), 71.7 ($\underline{C}HOC$), 72.9 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 77.8 ($\underline{C}HOC$), 78.0 ($\underline{C}HOC$), 100.5 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 169.6 (2x$CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{44}H_{74}NO_{18}$ [M-I$^-$]: 904.4900. Found: 904.4921.



220c

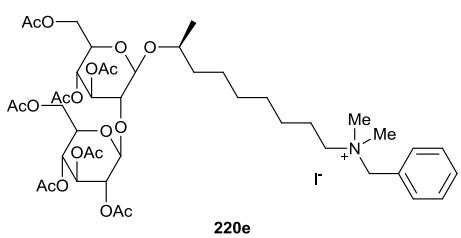
***N*,*N*,*N*-tributyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220d):** (0.56 g, 94%), brown-yellow powder, **mp** 67 °C. **IR (cm^{-1}) υ_{max}:** 730, 919, 1034 and 1064 (CHOCH), 1217 (COAc), 1366, 1743 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$): δ** 1.02 (9H, t, *J*=7.3 Hz, 3x$CH_2C\underline{H}_3$), 1.22 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.27-1.51 (15H, m, 3x$C\underline{H}_2CH_3$, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.53-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.68-1.77 (8H,



220d

m, 4xC$\underline{H}_2$CH$_2$N), 1.98 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (3H, s, C$\underline{H}_3$C=O), 2.09 (3H, s, C$\underline{H}_3$C=O), 3.31-3.35 (2H, m, CH$_2$C$\underline{H}_2$N), 3.37-3.42 (6H, m, 3xCH$_2$C$\underline{H}_2$N), 3.65-3.74 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.05-4.11 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.21-4.27 (2H, m, 2xCHCH$_a$$\underline{H}_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.71 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.87 (1H, dxd, *J*=9.5 Hz, *J*=8.2 Hz, C$\underline{H}$OC), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, C$\underline{H}$OC), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.12 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C$\underline{H}$OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC). **^{13}C-NMR (100MHz, CDCl$_3$):** δ 13.7 (3xCH$_2$C$\underline{H}_3$), 19.8 (3xC$\underline{H}_2$CH$_3$), 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.7 (C$\underline{H}_3$C=O), 20.7 (C$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 21.5 (C$\underline{H}_3$CH), 22.3 (C$\underline{H}_2$CH$_2$N), 24.3 (3xC$\underline{H}_2$CH$_2$N), 24.7 (C$\underline{H}_2$(CH$_2$)$_2$), 26.5 (C$\underline{H}_2$(CH$_2$)$_2$), 29.4 (C$\underline{H}_2$(CH$_2$)$_2$), 29.5 (C$\underline{H}_2$(CH$_2$)$_2$), 36.6 (C$\underline{H}_2$CHCH$_3$), 59.2 (3xCH$_2$C$\underline{H}_2$N), 59.3 (CH$_2$C$\underline{H}_2$N), 62.2 (C$\underline{H}_2$OAc), 62.4 (C$\underline{H}_2$OAc), 68.6 (C$\underline{H}$OC), 68.9 (C$\underline{H}$OC), 71.3 (C$\underline{H}$OC), 71.5 (C$\underline{H}$OC), 71.7 (C$\underline{H}$OC), 72.9 (C$\underline{H}$OC), 74.8 (C$\underline{H}$OC), 77.8 (C$\underline{H}$OC), 78.0 (C$\underline{H}$OC), 100.5 (C$\underline{H}$(O)$_2$), 101.3 (C$\underline{H}$(O)$_2$), 169.6 (CH$_3$C=O), 169.6 (CH$_3$C=O), 169.8 (CH$_3$C=O), 170.0 (2xCH$_3$C=O), 170.6 (CH$_3$C=O), 170.6 (CH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{47}H_{80}NO_{18}$ [M-I$^-$]: 946.5370. Found: 946.5374.

***N*-benzyl,*N*,*N*-dimethyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220e):** (0.45 g, quant.), off-white powder,

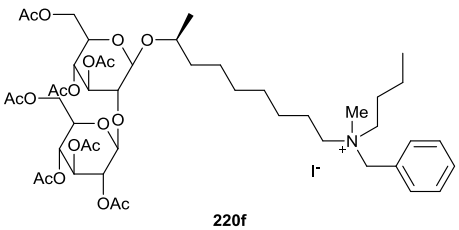


220e

mp 79 °C. **IR (cm^{-1}) υ$_{max}$:** 726, 914, 1033 and 1063 (CHOCH), 1217 (COAc), 1366, 1743 (C=O). **^{1}H-NMR (400MHz, CDCl$_3$):** δ 1.22 (3H, d, *J*=6.1 Hz, CHC$\underline{H}_3$), 1.26-1.44 (9H, m, 4xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.51-1.59 (1H, m, CH$_a$$\underline{H}_b$CHCH$_3$), 1.85-1.92 (2H, m, C$\underline{H}_2CH_2$N), 1.95 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.01 (3H, s, C$\underline{H}_3$C=O), 2.02 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.07 (3H, s, C$\underline{H}_3$C=O), 2.09 (3H, s, C$\underline{H}_3$C=O), 3.32 (6H, s, 2xC$\underline{H}_3$N), 3.50-3.54 (2H, m, CH$_2C\underline{H}_2$N), 3.65-3.74 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.05-4.11 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.27 (2H, m, 2xCHCH$_a$$\underline{H}_b$OAc), 4.47 (1H, d, J=7.6 Hz, C$\underline{H}$(O)$_2$), 4.71 (1H, d, J=8.0 Hz, C$\underline{H}$(O)$_2$), 4.84-4.89 (1H, m, C$\underline{H}$OC), 4.92 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.05 (2H, s, C$_{arom}$C$\underline{H}_2$N), 5.11 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC), 7.43-7.51 (3H, m, 3xC$\underline{H}_{arom}$), 7.69-7.70 (2H, m, 2xC$\underline{H}_{arom}$). **^{13}C-NMR (100MHz, CDCl$_3$):** δ 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (2xC$\underline{H}_3$C=O), 20.7 (C$\underline{H}_3$C=O), 20.8 (2xC$\underline{H}_3$C=O), 21.5 (C$\underline{H}_3$CH), 22.7 (C$\underline{H}_2$CH$_2$N), 24.6 (C$\underline{H}_2$(CH$_2$)$_2$), 26.2 (C$\underline{H}_2$(CH$_2$)$_2$), 29.3 (C$\underline{H}_2$(CH$_2$)$_2$), 29.4 (C$\underline{H}_2$(CH$_2$)$_2$), 36.5 (C$\underline{H}_2$CHCH$_3$), 49.8 (2xC$\underline{H}_3$N), 62.2 (C$\underline{H}_2$OAc), 62.3 (C$\underline{H}_2$OAc), 63.8 (CH$_2$C$\underline{H}_2$N), 67.0 (C$_{arom}$C$\underline{H}_2$N), 68.5 (C$\underline{H}$OC), 68.8 (C$\underline{H}$OC), 71.2 (C$\underline{H}$OC), 71.4 (C$\underline{H}$OC), 71.8 (C$\underline{H}$OC), 72.9 (C$\underline{H}$OC), 74.8 (C$\underline{H}$OC), 77.8 (C$\underline{H}$OC), 77.9 (C$\underline{H}$OC), 100.5 (C$\underline{H}$(O)$_2$), 101.2 (C$\underline{H}$(O)$_2$), 127.2 (C$_{arom}$), 129.2 (2xC$\underline{H}_{arom}$), 130.8 (C$\underline{H}_{arom}$), 133.2 (2xC$\underline{H}_{arom}$), 169.6 (2xCH$_3$C=O), 169.7 (CH$_3$C=O), 170.0 (2xCH$_3$C=O),

170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{44}H_{66}NO_{18}$ [M-I^-]: 896.4274. Found: 896.4270.

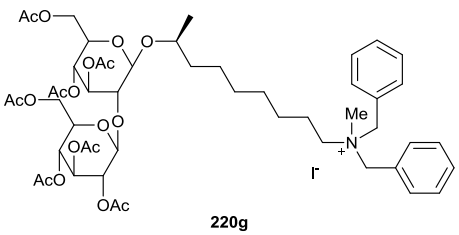
***N*-benzyl,*N*-butyl,*N*-methyl-(*(S)*-8-[(2",3',3",4',4",6',6"-hepta-acetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220f):** (0.12 g, 89%), light yellow powder,



220f

mp 76 °C. **IR (cm^{-1}) υ_{max}:** 727, 916, 1033 and 1062 (CHOCH), 1216 (COAc), 1366, 1742 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.01 (3H, t, *J*=7.3 Hz, $C\underline{H}_3CH_2$), 1.22 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.26-1.49 (11H, m, $C\underline{H}_2CH_3$, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.52-1.59 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.78-1.89 (4H, m, 2x$C\underline{H}_2CH_2N$), 1.94 (3H, s, $C\underline{H}_3C$=O), 2.00-2.02 (9H, m, 3x$C\underline{H}_3C$=O), 2.06-2.09 (9H, m, 3x$C\underline{H}_3C$=O), 3.22 (3H, s, $C\underline{H}_3N$), 3.32-3.41 (2H, m, $CH_2C\underline{H}_2N$), 3.43-3.59 (2H, m, $CH_2C\underline{H}_2N$), 3.65-3.76 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.05-4.11 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.21-4.27 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.85-5.02 (5H, m, 3x$C\underline{H}OC$, $C_{arom}C\underline{H}_2N$), 5.11 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 7.44-7.51 (3H, m, 3x$C\underline{H}_{arom}$), 7.66-7.69 (2H, m, 2x$C\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 13.7 ($\underline{C}H_3CH_2$), 19.7 ($CH_3\underline{C}H_2$), 20.5 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.5 ($\underline{C}H_3CH$), 22.6 ($\underline{C}H_2CH_2N$), 24.6 ($\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 47.8 ($\underline{C}H_3N$), 60.3 ($CH_2\underline{C}H_2N$), 60.4 ($CH_2\underline{C}H_2N$), 62.2 ($\underline{C}H_2OAc$), 62.4 ($\underline{C}H_2OAc$), 65.0 ($C_{arom}\underline{C}H_2N$), 68.5 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.2 ($\underline{C}HOC$), 71.4 ($\underline{C}HOC$), 71.7 ($\underline{C}HOC$), 72.9 ($\underline{C}HOC$), 74.8 ($\underline{C}HOC$), 77.7 ($\underline{C}HOC$), 78.0 ($\underline{C}HOC$), 100.5 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 127.0 ($\underline{C}_{arom}$), 129.3 (2x$\underline{C}H_{arom}$), 130.8 ($\underline{C}H_{arom}$), 133.2 (2x$\underline{C}H_{arom}$), 169.6 (2x$CH_3\underline{C}$=O), 169.8 ($CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{47}H_{72}NO_{18}$ [M-I^-]: 938.4744. Found: 938.4755.

***N*,*N*-dibenzyl,*N*-methyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220g):** (0.84 g, quant.), brown-yellow powder,

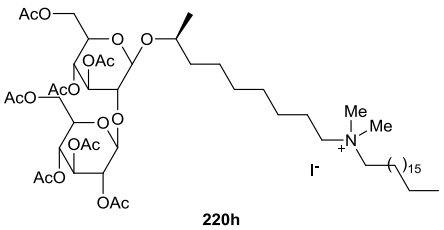


220g

mp 82 °C. **IR (cm^{-1}) υ_{max}:** 1037 (CHOCH), 1229 (COAc), 1367, 1749 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.23 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.26-1.47 (9H, m, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.52-1.58 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.91 (3H, s, $C\underline{H}_3C$=O), 1.95-2.09 (2H, m, $C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.07 (3H, s, $C\underline{H}_3C$=O), 2.09 (3H, s, $C\underline{H}_3C$=O), 3.15 (3H, s, $C\underline{H}_3N$), 3.21-3.25 (2H, m, $CH_2C\underline{H}_2N$), 3.66-3.76 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.06-4.11 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.22-4.28 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.46 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.70 (1H,

d, *J*=8.0 Hz, C<u>H</u>$(O)_2$), 4.86-5.20 (9H, m, 5xC<u>H</u>OC, 2x$C_{arom}C\underline{H}_2$N), 7.45-7.69 (10H, m, 10xC<u>H</u>$_{arom}$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.4 (<u>C</u>H_3C=O), 20.6 (2x<u>C</u>H_3C=O), 20.6 (<u>C</u>H_3C=O), 20.7 (<u>C</u>H_3C=O), 20.8 (<u>C</u>H_3C=O), 20.8 (<u>C</u>H_3C=O), 21.4 (<u>C</u>H_3CH), 22.8 (<u>C</u>H_2CH_2N), 24.6 (<u>C</u>$H_2(CH_2)_2$), 26.4 (<u>C</u>$H_2(CH_2)_2$), 29.3 (<u>C</u>$H_2(CH_2)_2$), 29.4 (<u>C</u>$H_2(CH_2)_2$), 36.4 (<u>C</u>H_2CHCH$_3$), 46.5 (<u>C</u>H_3N), 59.3 (CH_2<u>C</u>H_2N), 62.2 (<u>C</u>H_2OAc), 62.3 (<u>C</u>H_2OAc), 64.5 (2x$C_{arom}\underline{C}H_2$N), 68.5 (<u>C</u>HOC), 68.8 (<u>C</u>HOC), 71.2 (<u>C</u>HOC), 71.4 (<u>C</u>HOC), 71.6 (<u>C</u>HOC), 72.9 (<u>C</u>HOC), 74.7 (<u>C</u>HOC), 77.7 (<u>C</u>HOC), 77.9 (<u>C</u>HOC), 100.4 (<u>C</u>H$(O)_2$), 101.1 (<u>C</u>H$(O)_2$), 127.1 (2x<u>C</u>$_{arom}$), 129.3 (4x<u>C</u>H$_{arom}$), 130.7 (2x<u>C</u>H$_{arom}$), 133.2 (4x<u>C</u>H$_{arom}$), 169.6 (CH_3<u>C</u>=O), 169.6 (CH_3<u>C</u>=O), 169.7 (CH_3<u>C</u>=O), 170.0 (2xCH_3<u>C</u>=O), 170.5 (CH_3<u>C</u>=O), 170.6 (CH_3<u>C</u>=O). **MS (ESI): m/z** Exact mass calculated for $C_{50}H_{70}NO_{18}$ [M-I$^-$]: 972.4587. Found: 972.4574.

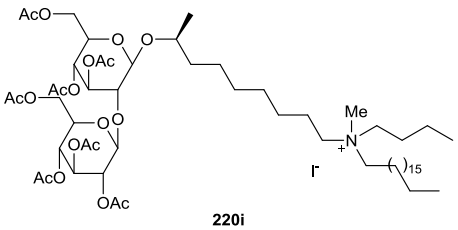
***N*,*N*-dimethyl,*N*-octadecyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220h):** (0.51 g, 98%), orange-yellow powder,



220h

mp 50 °C. **IR (cm^{-1}) υ_{max}:** 729, 918, 1034 and 1063 (CHOCH), 1217 (COAc), 1366, 1746 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.88 (3H, t, *J*=6.7 Hz, C<u>H</u>$_3$CH$_2$), 1.22 (3H, d, *J*=6.2 Hz, C<u>H</u>$_3$CH), 1.26-1.46 (39H, m, C<u>H</u>$_2$CH$_3$, 18xC<u>H</u>$_2(CH_2)_2$, C<u>H</u>$_a$H$_b$CHCH$_3$), 1.51-1.58 (1H, m, CH$_a$<u>H</u>$_b$CHCH$_3$), 1.71-1.82 (4H, m, 2xC<u>H</u>$_2$CH$_2$N), 1.99 (3H, s, C<u>H</u>$_3$C=O), 2.00 (3H, s, C<u>H</u>$_3$C=O), 2.03 (6H, s, 2xC<u>H</u>$_3$C=O), 2.06 (3H, s, C<u>H</u>$_3$C=O), 2.08 (3H, s, C<u>H</u>$_3$C=O), 2.09 (3H, s, C<u>H</u>$_3$C=O), 3.39 (6H, s, 2xC<u>H</u>$_3$N), 3.46-3.50 (2H, m, CH$_2$C<u>H</u>$_2$N), 3.55-3.59 (2H, m, CH$_2$C<u>H</u>$_2$N), 3.65-3.74 (4H, m, 2xC<u>H</u>CH$_2$OAc, CH$_3$C<u>H</u>O, C<u>H</u>OC), 4.05-4.11 (2H, m, 2xCHC<u>H</u>$_a$H$_b$OAc), 4.22-4.27 (2H, m, 2xCHCH$_a$<u>H</u>$_b$OAc), 4.47 (1H, d, *J*=7.6 Hz, C<u>H</u>$(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, C<u>H</u>$(O)_2$), 4.84-4.89 (1H, m, C<u>H</u>OC), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, C<u>H</u>OC), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C<u>H</u>OC), 5.12 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C<u>H</u>OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C<u>H</u>OC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 14.1 (<u>C</u>H_3CH$_2$), 20.5 (<u>C</u>H_3C=O), 20.6 (<u>C</u>H_3C=O), 20.7 (2x<u>C</u>H_3C=O), 20.7 (<u>C</u>H_3C=O), 20.8 (2x<u>C</u>H_3C=O), 21.5 (<u>C</u>H_3CH), 22.6 (<u>C</u>H_2CH_2N), 22.7 (<u>C</u>H_2CH_2N), 22.9 (CH_3<u>C</u>H_2), 24.6 (<u>C</u>$H_2(CH_2)_2$), 26.2 (<u>C</u>$H_2(CH_2)_2$), 26.2 (<u>C</u>$H_2(CH_2)_2$), 29.3 (<u>C</u>$H_2(CH_2)_2$), 29.3 (<u>C</u>$H_2(CH_2)_2$), 29.3 (<u>C</u>$H_2(CH_2)_2$), 29.4 (<u>C</u>$H_2(CH_2)_2$), 29.4 (<u>C</u>$H_2(CH_2)_2$), 29.5 (<u>C</u>$H_2(CH_2)_2$), 29.6-29.7 (8x<u>C</u>$H_2(CH_2)_2$), 31.9 (<u>C</u>$H_2(CH_2)_2$), 36.5 (<u>C</u>H_2CHCH$_3$), 51.4 (2x<u>C</u>H_3N), 62.2 (<u>C</u>H_2OAc), 62.3 (<u>C</u>H_2OAc), 64.2 (2xCH_2<u>C</u>H_2N), 68.5 (<u>C</u>HOC), 68.8 (<u>C</u>HOC), 71.2 (<u>C</u>HOC), 71.4 (<u>C</u>HOC), 71.7 (<u>C</u>HOC), 72.9 (<u>C</u>HOC), 74.8 (<u>C</u>HOC), 77.8 (<u>C</u>HOC), 78.0 (<u>C</u>HOC), 100.5 (<u>C</u>H$(O)_2$), 101.3 (<u>C</u>H$(O)_2$), 169.6 (2xCH_3<u>C</u>=O), 169.7 (CH_3<u>C</u>=O), 170.0 (2xCH_3<u>C</u>=O), 170.5 (CH_3<u>C</u>=O), 170.6 (CH_3<u>C</u>=O). **MS (ESI): m/z** Exact mass calculated for $C_{55}H_{96}NO_{18}$ [M-I$^-$]: 1058.6622. Found: 1058.6611.

***N*-butyl,*N*-methyl,*N*-octadecyl-((*S*)-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (220i):** (0.59 g, quant.), viscous yellow oil.



220i

IR (cm^{-1}) υ_{max}: 727, 909, 1035 and 1064 (CHOCH), 1222 (COAc), 1366, 1747 (C=O). **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 0.88 (3H, t, *J*=6.8 Hz, C$\underline{H}_3$CH$_2$), 1.02 (3H, t, *J*=7.3 Hz, C$\underline{H}_3$CH$_2$), 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.26-1.52 (41H, m, 2xC$\underline{H}_2$CH$_3$, 18xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.54-1.62 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.68-1.79 (6H, m, 3xC$\underline{H}_2$CH$_2$N), 1.99 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (3H, s, C$\underline{H}_3$C=O), 2.09 (3H, s, C$\underline{H}_3$C=O), 3.31 (3H, s, C$\underline{H}_3$N), 3.38-3.53 (6H, m, 3xCH$_2$C$\underline{H}_2$N), 3.65-3.74 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.05-4.11 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.27 (2H, m, 2xCHCH$_a\underline{H}_b$OAc), 4.47 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.71 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.87 (1H, dxd, *J*=9.6 Hz, *J*=8.1 Hz, C$\underline{H}$OC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.00 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, C$\underline{H}$OC), 5.12 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C$\underline{H}$OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 13.7 (C$\underline{H}_3$CH$_2$), 14.1 (C$\underline{H}_3$CH$_2$), 19.7 (CH$_3$C$\underline{H}_2$), 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6 (2xC$\underline{H}_3$C=O), 20.7 (C$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 20.8 (C$\underline{H}_3$C=O), 21.5 (C$\underline{H}_3$CH), 22.4 (C$\underline{H}_2$CH$_2$N), 22.5 (C$\underline{H}_2$CH$_2$N), 22.6 (CH$_3$C$\underline{H}_2$), 24.4 (C$\underline{H}_2$CH$_2$N), 24.7 (C$\underline{H}_2$(CH$_2$)$_2$), 26.3 (C$\underline{H}_2$(CH$_2$)$_2$), 26.4 (C$\underline{H}_2$(CH$_2$)$_2$), 29.2-29.7 (14xC$\underline{H}_2$(CH$_2$)$_2$), 31.9 (C$\underline{H}_2$(CH$_2$)$_2$), 36.5 (C$\underline{H}_2$CHCH$_3$), 49.1 (C$\underline{H}_3$N), 61.6 (2xCH$_2$C$\underline{H}_2$N), 61.9 (CH$_2$C$\underline{H}_2$N), 62.2 (C$\underline{H}_2$OAc), 62.4 (C$\underline{H}_2$OAc), 68.5 (C$\underline{H}$OC), 68.8 (C$\underline{H}$OC), 71.2 (C$\underline{H}$OC), 71.4 (C$\underline{H}$OC), 71.8 (C$\underline{H}$OC), 72.9 (C$\underline{H}$OC), 74.8 (C$\underline{H}$OC), 77.8 (C$\underline{H}$OC), 78.0 (C$\underline{H}$OC), 100.5 (C$\underline{H}$(O)$_2$), 101.3 (C$\underline{H}$(O)$_2$), 169.6 (2xCH$_3$C$\underline{=}$O), 169.8 (CH$_3$C$\underline{=}$O), 170.0 (2xCH$_3$C$\underline{=}$O), 170.6 (CH$_3$C$\underline{=}$O), 170.6 (CH$_3$C$\underline{=}$O). **MS (ESI): m/z** Exact mass calculated for $C_{58}H_{102}NO_{18}$ [M-I$^-$]: 1100.7091. Found: 1100.7077.

***N*,*N*-dimethyl,*N*-octadecyl-((*S*)-11-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])dodecan-1-ammonium iodide (227a):** (0.57 g, 99%), orange-yellow powder.

227a

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 0.88 (3H, t, *J*=6.8 Hz, C$\underline{H}_3$CH$_2$), 1.22 (3H, d, *J*=6.2 Hz, C$\underline{H}_3$CH), 1.24-1.44 (45H, m, C$\underline{H}_2$CH$_3$, 21xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.53-1.63 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.69-1.78 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 1.99 (3H, s, C$\underline{H}_3$C=O), 2.00 (3H, s, C$\underline{H}_3$C=O), 2.03 (3H, s, C$\underline{H}_3$C=O), 2.04 (3H, s, C$\underline{H}_3$C=O), 2.06 (3H, s, C$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 3.40 (6H, s, 2xC$\underline{H}_3$N), 3.48-3.57 (4H, m, 2xCH$_2$C$\underline{H}_2$N), 3.64-3.76 (4H, m, 2xC$\underline{H}$CH$_2$OAc, CH$_3$C$\underline{H}$O, C$\underline{H}$OC), 4.05-4.10 (2H, m, 2xCHC$\underline{H}_a$H$_b$OAc), 4.23-4.30 (2H, m, 2xCHCH$_a\underline{H}_b$OAc), 4.48 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.74 (1H, d, *J*=8.0 Hz, C$\underline{H}$(O)$_2$), 4.90 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, C$\underline{H}$OC), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, C$\underline{H}$OC), 5.05 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, C$\underline{H}$OC), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, C$\underline{H}$OC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, C$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 14.1 (C$\underline{H}_3$CH$_2$), 20.5 (C$\underline{H}_3$C=O), 20.6 (C$\underline{H}_3$C=O), 20.6

(2x$\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.2 ($\underline{C}H_3CH$), 22.6 ($CH_3\underline{C}H_2$), 22.7 ($CH_2\underline{C}H_2N$), 22.8 ($CH_2\underline{C}H_2N$), 24.9 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.6 (10x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 36.4 ($\underline{C}H_2CHCH_3$), 51.5 (2x$\underline{C}H_3N$), 62.0 ($\underline{C}H_2OAc$), 62.2 ($\underline{C}H_2OAc$), 64.1 (2x$CH_2\underline{C}H_2N$), 68.2 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.2 ($\underline{C}HOC$), 71.6 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.6 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 77.9 ($\underline{C}HOC$), 100.3 ($\underline{C}H(O)_2$), 101.1 ($\underline{C}H(O)_2$), 169.4 ($CH_3\underline{C}$=O), 169.5 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.2 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O).

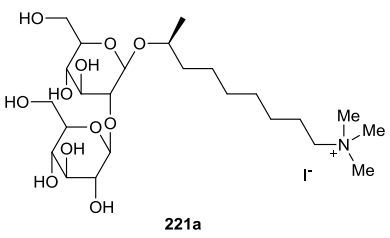
***N*-butyl,*N*-methyl,*N*-octadecyl-(*(S)*-11-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])dodecan-1-ammonium iodide (227b):** (0.57 g, 97%), viscous orange-yellow oil. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.88 (3H, t, *J*=6.8 Hz, $C\underline{H}_3CH_2$), 1.02 (3H, t, *J*=7.3 Hz, $C\underline{H}_3CH_2$), 1.22 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.24-1.52 (47H, m, 2x$C\underline{H}_2CH_3$, 21x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.55-1.63 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.67-1.75 (6H, m, 3x$C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.00 (3H, s, $C\underline{H}_3C$=O), 2.02 (3H, s, $C\underline{H}_3C$=O), 2.04 (3H, s, $C\underline{H}_3C$=O), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.08 (6H, s, 2x$C\underline{H}_3C$=O), 3.33 (3H, s, $C\underline{H}_3N$), 3.40-3.52 (6H, m, 3x$CH_2C\underline{H}_2N$), 3.64-3.75 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.05-4.10 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.23-4.30 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.48 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.73 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.90 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, $C\underline{H}OC$), 5.05 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, $C\underline{H}OC$), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$).

227b

^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 13.8 ($\underline{C}H_3CH_2$), 14.1 ($\underline{C}H_3CH_2$), 19.7 ($CH_3\underline{C}H_2$), 20.5 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.3 ($\underline{C}H_3CH$), 22.5 ($CH_2\underline{C}H_2N$), 22.5 ($CH_2\underline{C}H_2N$), 22.7 ($CH_3\underline{C}H_2$), 24.4 ($CH_2\underline{C}H_2N$), 25.0 ($\underline{C}H_2(CH_2)_2$), 26.3 (2x$\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.3 (2x$\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.5 (2x$\underline{C}H_2(CH_2)_2$), 29.6 ($\underline{C}H_2(CH_2)_2$), 29.6-29.7 (9x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 49.2 ($\underline{C}H_3N$), 61.6 ($CH_2\underline{C}H_2N$), 61.7 ($CH_2\underline{C}H_2N$), 61.8 ($CH_2\underline{C}H_2N$), 62.1 ($\underline{C}H_2OAc$), 62.2 ($\underline{C}H_2OAc$), 68.3 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.2 ($\underline{C}HOC$), 71.6 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.6 ($\underline{C}HOC$), 77.7 ($\underline{C}HOC$), 77.9 ($\underline{C}HOC$), 100.4 ($\underline{C}H(O)_2$), 101.1 ($\underline{C}H(O)_2$), 169.4 ($CH_3\underline{C}$=O), 169.5 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.2 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O).

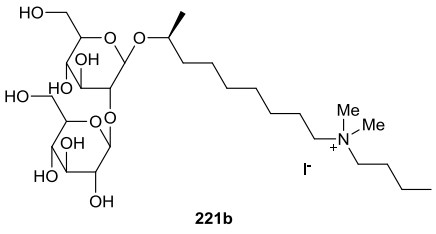
General procedure for the synthesis of deprotected sophorolipid quaternary ammonium salts: In a 25 mL flame dried round-bottomed flask, the peracetylated sophorolipid quaternary ammonium salt was dissolved in dry methanol and sodium methoxide (0.15 eq) was added. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and recrystallized from acetone to yield the pure deprotected sophorolipid quaternary ammonium salt.

***N*,*N*,*N*-trimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221a):** (0.30 g, quant.), off-white powder, **mp** 119 °C. **IR (cm^{-1}) υ_{max}:** 726, 914, 1022 and 1068 (CHOCH), 3358 (OH). **^{1}H-NMR (400MHz, MeOD):** δ 1.16 (3H, d, *J*=6.3 Hz, $CHC\underline{H}_3$), 1.19-1.42 (9H, m, $4xC\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.46-1.54 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.67-1.75 (2H, m, $3xC\underline{H}_2CH_2N$), 3.04 (9H, s, $3xC\underline{H}_3N$), 3.09-3.31 (8H, m, $CH_2C\underline{H}_2N$, 6xC<u>H</u>OC), 3.39 (1H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, C<u>H</u>OC), 3.46 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, C<u>H</u>OC), 3.53-3.58 (2H, m, $2xCHC\underline{H}_aH_bOH$), 3.70-3.78 (3H, m, $2xCHCH_a\underline{H}_bOH$, C<u>H</u>OC), 4.35 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.59 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100MHz, MeOD):** δ 20.7 ($CHC\underline{H}_3$), 22.6 ($\underline{C}H_2CH_2N$), 24.6 ($\underline{C}H_2(CH_2)_2$), 25.9 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 52.2 ($3x\underline{C}H_3N$), 61.4 ($\underline{C}H_2OH$), 61.6 ($\underline{C}H_2OH$), 66.5 ($CH_2\underline{C}H_2N$), 70.2 (<u>C</u>HOC), 70.4 (<u>C</u>HOC), 74.7 (<u>C</u>HOC), 76.4 ($2x\underline{C}HOC$), 76.8 (<u>C</u>HOC), 77.0 (<u>C</u>HOC), 77.7 (<u>C</u>HOC), 79.8 (<u>C</u>HOC), 101.6 ($\underline{C}H(O)_2$), 103.0 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{24}H_{48}NO_{11}$ [M-I^-]: 526.3222. Found: 526.3218.



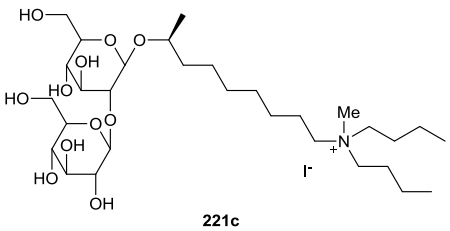
221a

***N*-butyl,*N*,*N*-dimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221b):** (0.34 g, 88%), white powder, **mp** 70 °C. **IR (cm^{-1}) υ_{max}:** 726, 908, 1030 and 1069 (CHOCH), 3348 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.93 (3H, t, *J*=7.4 Hz, $CH_2C\underline{H}_3$), 1.16 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.19-1.44 (11H, m, $C\underline{H}_2CH_3$, $4xC\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.46-1.54 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.61-1.72 (4H, m, $2xC\underline{H}_2CH_2N$), 2.98 (6H, s, $2xC\underline{H}_3N$), 3.10-3.24 (9H, m, $2xCH_2C\underline{H}_2N$, 5xC<u>H</u>OC), 3.29 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, C<u>H</u>OC), 3.38 (1H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, C<u>H</u>OC), 3.46 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, C<u>H</u>OC), 3.53-3.58 (2H, m, $2xCHC\underline{H}_aH_bOH$), 3.70-3.78 (3H, m, $2xCHCH_a\underline{H}_bOH$, C<u>H</u>OC), 4.35 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.58 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.6 ($CH_2C\underline{H}_3$), 19.3 ($C\underline{H}_2CH_3$), 20.7 ($CHC\underline{H}_3$), 22.2 ($\underline{C}H_2CH_2N$), 24.1 ($\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.0 ($\underline{C}H_2(CH_2)_2$), 29.0 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 49.8 ($2xCH_3N$), 61.4 ($\underline{C}H_2OH$), 61.6 ($\underline{C}H_2OH$), 63.9 ($CH_2\underline{C}H_2N$), 64.1 ($CH_2\underline{C}H_2N$), 70.2 (<u>C</u>HOC), 70.5 (<u>C</u>HOC), 74.7 (<u>C</u>HOC), 76.4 ($2x\underline{C}HOC$), 76.8 (<u>C</u>HOC), 77.0 (<u>C</u>HOC), 77.6 (<u>C</u>HOC), 80.0 (<u>C</u>HOC), 101.6 ($\underline{C}H(O)_2$), 103.0 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{27}H_{54}NO_{11}$ [M-I^-]: 568.3691. Found: 568.3689.



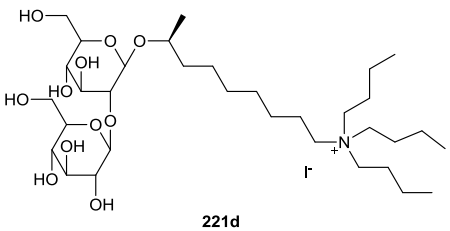
221b

***N*,*N*-dibutyl,*N*-methyl-((*S*)-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (2218c):** (0.43 g, quant.), white powder, **mp** 94 °C. **IR (cm^{-1}) υ_{max}:** 725, 916, 1024 and 1069 (CHOCH), 3344 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.92 (6H, t, *J*=7.4 Hz, 2x$CH_2C\underline{H}_3$), 1.14 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.20-1.42 (13H, m, 2x$C\underline{H}_2CH_3$, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.45-1.53 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.57-1.67 (6H, m, 3x$C\underline{H}_2CH_2N$), 2.93 (3H, s, $C\underline{H}_3N$), 3.09-3.24 (11H, m, 3x$CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.28 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, $C\underline{H}OC$), 3.35 (1H, dxd, *J*=9.0 Hz, *J*=7.9 Hz, $C\underline{H}OC$), 3.46 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $C\underline{H}OC$), 3.52-3.57 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.69-3.76 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.34 (1H, d, *J*=7.7 Hz, $C\underline{H}(O)_2$), 4.55 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.6 (2x$CH_2\underline{C}H_3$), 19.4 (2x$\underline{C}H_2CH_3$), 20.6 ($CH\underline{C}H_3$), 21.9 ($\underline{C}H_2CH_2N$), 23.8 (2x$\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 28.9 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 47.5 ($\underline{C}H_3N$), 61.2 (2x$CH_2\underline{C}H_2N$), 61.4, 61.5, 61.7 (2x$\underline{C}H_2OH$, $CH_2\underline{C}H_2N$), 70.3 ($\underline{C}HOC$), 70.5 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 76.4 ($\underline{C}HOC$), 76.5 ($\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.0 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 80.3 ($\underline{C}HOC$), 101.5 ($\underline{C}H(O)_2$), 103.3 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{30}H_{60}NO_{11}$ [M-I^-]: 610.4161. Found: 610.4162.



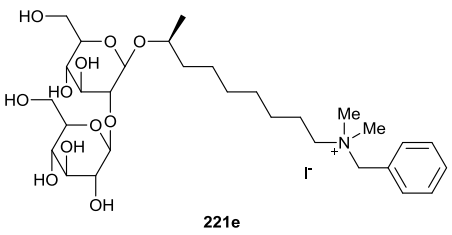
221c

***N*,*N*,*N*-tributyl-((*S*)-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221d):** (0.22 g, quant.), off-white powder, **mp** 100 °C. **IR (cm^{-1}) υ_{max}:** 724, 906, 1027 and 1072 (CHOCH), 3370 (OH). **^{1}H-NMR (400MHz, MeOD):** δ 0.93 (9H, t, *J*=7.4 Hz, 3x$CH_2C\underline{H}_3$), 1.16 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.19-1.44 (15H, m, 3x$C\underline{H}_2CH_3$, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.46-1.52 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.53-1.63 (8H, m, 3x$C\underline{H}_2CH_2N$), 3.10-3.23 (13H, m, 4x$CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.29 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, $C\underline{H}OC$), 3.37 (1H, dxd, *J*=9.1 Hz, *J*=7.7 Hz, $C\underline{H}OC$), 3.46 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $C\underline{H}OC$), 3.53-3.58 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.70-3.78 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.35 (1H, d, *J*=7.7 Hz, $C\underline{H}(O)_2$), 4.56 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100MHz, MeOD):** δ 12.6 (3x$CH_2\underline{C}H_3$), 19.3 (3x$\underline{C}H_2CH_3$), 20.6 ($CH\underline{C}H_3$), 21.5 ($\underline{C}H_2CH_2N$), 23.5 (3x$\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 28.9 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 36.4 ($\underline{C}H_2CHCH_3$), 58.1 (3x$CH_2\underline{C}H_2N$), 58.4 ($CH_2\underline{C}H_2N$), 61.4 ($\underline{C}H_2OH$), 61.6 ($\underline{C}H_2OH$), 70.2 ($\underline{C}HOC$), 70.5 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 76.4 (2x$\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.5 ($\underline{C}HOC$), 80.2 ($\underline{C}HOC$), 101.5 ($\underline{C}H(O)_2$), 103.2 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{33}H_{66}NO_{11}$ [M-I^-]: 652.4630. Found: 652.4618.



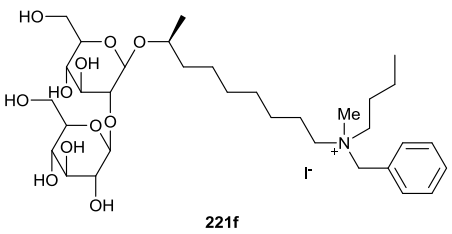
221d

***N*-benzyl,*N*,*N*-dimethyl-((*S*)-8-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221e):** (0.35 g, quant.), white powder, **mp** 76 °C. **IR (cm^{-1}) υ_{max}:** 726, 916, 1028 and 1065 (CHOCH), 3360 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.16 (3H, d, *J*=6.2 Hz, CHC$\underline{H}_3$), 1.22-1.42 (9H, m, 4xC$\underline{H}_2(CH_2)_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.47-1.55 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.78-1.86 (2H, m, C$\underline{H}_2$CH$_2$N), 2.94 (6H, s, 2xC$\underline{H}_3$N), 3.10-3.31 (8H, m, CH$_2$C$\underline{H}_2$N, 6xC$\underline{H}$OC), 3.39 (1H, dxd, *J*=9.1 Hz, *J*=7.7 Hz, C$\underline{H}$OC), 3.46 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, C$\underline{H}$OC), 3.54-3.58 (2H, m, 2xCHC$\underline{H}_a$H$_b$OH), 3.70-3.78 (3H, m, 2xCHCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.35 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.45 (2H, s, C$_{arom}$C$\underline{H}_2$N), 4.59 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$), 7.42-7.50 (5H, m, 5xC$\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.7 (CH$\underline{C}$H$_3$), 22.3 ($\underline{C}$H$_2$CH$_2$N), 24.6 7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 26.0 7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 ($\underline{C}$H$_2$CHCH$_3$), 49.0 (2xCH$_3$N), 61.4 ($\underline{C}$H$_2$OH), 61.7 ($\underline{C}$H$_2$OH), 64.6 (CH$_2\underline{C}$H$_2$N), 67.4 (C$_{arom}\underline{C}$H$_2$N), 70.2 ($\underline{C}$HOC), 70.5 ($\underline{C}$HOC), 74.7 ($\underline{C}$HOC), 76.4 ($\underline{C}$HOC), 76.5 ($\underline{C}$HOC), 76.9 ($\underline{C}$HOC), 77.0 ($\underline{C}$HOC), 77.7 ($\underline{C}$HOC), 80.0 ($\underline{C}$HOC), 101.6 ($\underline{C}$H(O)$_2$), 103.1 ($\underline{C}$H(O)$_2$), 127.6 ($\underline{C}_{arom}$), 129.0 (2x$\underline{C}$H$_{arom}$), 130.5 0 ($\underline{C}$H$_{arom}$), 132.7 0 (2x$\underline{C}$H$_{arom}$). **MS (ESI): m/z** Exact mass calculated for $C_{30}H_{52}NO_{11}$ [M-I$^-$]: 602.3535. Found: 602.3527.



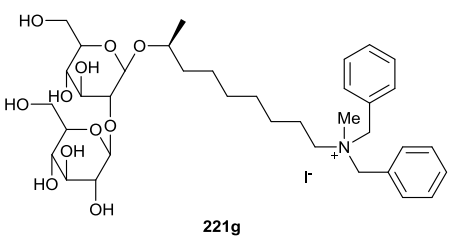
221e

***N*-benzyl,*N*-butyl,*N*-methyl-((*S*)-8-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221f):** (0.31 g, quant.), white powder, **mp** 112 °C. **IR (cm^{-1}) υ_{max}:** 725, 908, 1025 and 1071 (CHOCH), 3356 (OH). **^{1}H-NMR (400MHz, MeOD):** δ 0.94 (3H, t, *J*=7.4 Hz, CH$_2$C$\underline{H}_3$), 1.16 (3H, d, *J*=6.2 Hz, CHC$\underline{H}_3$), 1.20-1.44 (11H, m, C$\underline{H}_2$CH$_3$, 4xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.47-1.55 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.67-1.82 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 2.88 (3H, s, C$\underline{H}_3$N), 3.10-3.23 (9H, m, 2xCH$_2$C$\underline{H}_2$N, 5xC$\underline{H}$OC), 3.29 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, C$\underline{H}$OC), 3.38 (1H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, C$\underline{H}$OC), 3.46 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, C$\underline{H}$OC), 3.53-3.58 (2H, m, 2xCHC$\underline{H}_a$H$_b$OH), 3.71-3.78 (3H, m, 2xCHCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.35 (1H, d, *J*=7.6 Hz, C$\underline{H}$(O)$_2$), 4.45 (2H, s, C$_{arom}$C$\underline{H}_2$N), 4.58 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$), 7.44-7.47 (5H, m, 5xC$\underline{H}_{arom}$). **^{13}C-NMR (100MHz, MeOD):** δ 12.6 (CH$_2\underline{C}$H$_3$), 19.3 ($\underline{C}$H$_2$CH$_3$), 20.6 (CH$\underline{C}$H$_3$), 22.0 ($\underline{C}$H$_2$CH$_2$N), 23.9 ($\underline{C}$H$_2$CH$_2$N), 24.7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 26.1 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 ($\underline{C}$H$_2$(CH$_2$)$_2$), 36.4 ($\underline{C}$H$_2$CHCH$_3$), 46.9 ($\underline{C}$H$_3$N), 60.6 (CH$_2\underline{C}$H$_2$N), 61.0 (CH$_2\underline{C}$H$_2$N), 61.4 ($\underline{C}$H$_2$OH), 61.6 ($\underline{C}$H$_2$OH), 65.3 (C$_{arom}\underline{C}$H$_2$N), 70.2 ($\underline{C}$HOC), 70.5 ($\underline{C}$HOC), 74.7 ($\underline{C}$HOC), 76.4 (2x$\underline{C}$HOC), 76.9 ($\underline{C}$HOC), 77.0 ($\underline{C}$HOC), 77.6 ($\underline{C}$HOC), 80.0 ($\underline{C}$HOC), 101.6 ($\underline{C}$H(O)$_2$), 103.1 ($\underline{C}$H(O)$_2$), 127.5 ($\underline{C}_{arom}$), 129.0 (2x$\underline{C}$H$_{arom}$), 130.5 ($\underline{C}$H$_{arom}$), 132.7 (2x$\underline{C}$H$_{arom}$). **MS (ESI): m/z** Exact mass calculated for $C_{33}H_{58}NO_{11}$ [M-I$^-$]: 644.4004. Found: 644.3998.

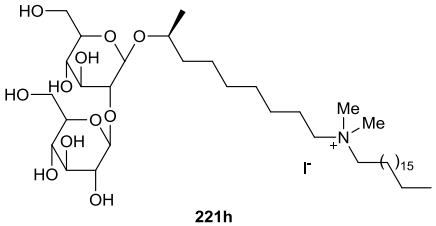


221f

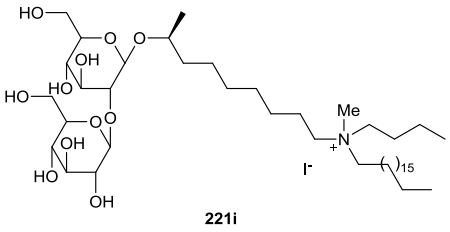
***N*,*N*-dibenzyl,*N*-methyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221g):** (0.24 g, 99%), light brown powder, **mp** 158 °C. **IR (cm^{-1}) υ_{max}:** 702, 722, 918, 1025 (CHOCH), 3350 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.16 (3H, d, *J*=6.2 Hz, CHC$\underline{H}_3$), 1.23-1.44 (9H, m, 4xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.47-1.55 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.87-1.95 (2H, m, C$\underline{H}_2$CH$_2$N), 2.84 (3H, s, C$\underline{H}_3$N), 3.07-3.22 (7H, m, CH$_2$C$\underline{H}_2$N, 5xC$\underline{H}$OC), 3.29 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, C$\underline{H}$OC), 3.36-3.40 (1H, m, C$\underline{H}$OC), 3.46 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, C$\underline{H}$OC), 3.53-3.58 (2H, m, 2xCHC$\underline{H}_a$H$_b$OH), 3.71-3.78 (3H, m, 2xCHCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.35 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.44 (2H, d, *J*=12.9 Hz, 2xC$_{arom}$C$\underline{H}_a$H$_b$N), 4.57 (2H, d, *J*=12.9 Hz, 2xC$_{arom}$CH$_a\underline{H}_b$N), 4.58 (1H, d, *J*=8.1 Hz, C$\underline{H}$(O)$_2$), 70.43-70.50 (10H, m, 10xC$\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.6 (CH$\underline{C}$H$_3$), 22.2 ($\underline{C}$H$_2$CH$_2$N), 24.6 ($\underline{C}$H$_2$(CH$_2$)$_2$), 26.0 ($\underline{C}$H$_2$(CH$_2$)$_2$), 28.9 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 ($\underline{C}$H$_2$(CH$_2$)$_2$), 36.4 ($\underline{C}$H$_2$CHCH$_3$), 46.2 ($\underline{C}$H$_3$N), 60.7 (CH$_2\underline{C}$H$_2$N), 61.4 ($\underline{C}$H$_2$OH), 61.7 ($\underline{C}$H$_2$OH), 65.5 (2xC$_{arom}\underline{C}$H$_2$N), 70.2 ($\underline{C}$HOC), 70.5 ($\underline{C}$HOC), 74.7 ($\underline{C}$HOC), 76.4 ($\underline{C}$HOC), 76.5 ($\underline{C}$HOC), 76.9 ($\underline{C}$HOC), 77.0 ($\underline{C}$HOC), 77.6 ($\underline{C}$HOC), 80.1 ($\underline{C}$HOC), 101.6 ($\underline{C}$H(O)$_2$), 103.2 ($\underline{C}$H(O)$_2$), 127.4 (2x$\underline{C}_{arom}$), 129.1 (4x$\underline{C}$H$_{arom}$), 130.5 (2x$\underline{C}$H$_{arom}$), 132.9 (4x$\underline{C}$H$_{arom}$). **MS (ESI): m/z** Exact mass calculated for $C_{36}H_{56}NO_{11}$ [M-I$^-$]: 678.3848. Found: 678.3843.



***N*,*N*-dimethyl,*N*-octadecyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221h):** (0.22 g, 97%), white powder, **mp** 114 °C. **IR (cm^{-1}) υ_{max}:** 728, 906, 1073 (CHOCH), 3360 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.79 (3H, t, *J*=6.8 Hz, CH$_2$C$\underline{H}_3$), 1.14 (3H, d, *J*=6.2 Hz, CHC$\underline{H}_3$), 1.18-1.43 (39H, m, C$\underline{H}_2$CH$_3$, 18xC$\underline{H}_2$(CH$_2$)$_2$, C$\underline{H}_a$H$_b$CHCH$_3$), 1.44-1.53 (1H, m, CH$_a\underline{H}_b$CHCH$_3$), 1.61-1.70 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 2.97 (6H, s, 2xC$\underline{H}_3$N), 3.08-3.24 (9H, m, 2xCH$_2$C$\underline{H}_2$N, 5xC$\underline{H}$OC), 3.28 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, C$\underline{H}$OC), 3.36 (1H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, C$\underline{H}$OC), 3.45 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, C$\underline{H}$OC), 3.52-3.57 (2H, m, 2xCHC$\underline{H}_a$H$_b$OH), 3.68-3.76 (3H, m, 2xCHCH$_a\underline{H}_b$OH, C$\underline{H}$OC), 4.33 (1H, d, *J*=7.7 Hz, C$\underline{H}$(O)$_2$), 4.57 (1H, d, *J*=7.8 Hz, C$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 13.1 (CH$_2\underline{C}$H$_3$), 20.7 (CH$\underline{C}$H$_3$), 22.2 ($\underline{C}$H$_2$CH$_2$N), 22.2 ($\underline{C}$H$_2$CH$_2$N), 22.3 ($\underline{C}$H$_2$CH$_3$), 24.7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 26.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 28.8 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.1 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.2 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.2 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 ($\underline{C}$H$_2$(CH$_2$)$_2$), 29.3-29.4 (8x$\underline{C}$H$_2$(CH$_2$)$_2$), 31.7 ($\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 ($\underline{C}$H$_2$CHCH$_3$), 49.8 (2x$\underline{C}$H$_3$N), 61.4 ($\underline{C}$H$_2$OH), 61.7 ($\underline{C}$H$_2$OH), 64.1 (2xCH$_2\underline{C}$H$_2$N), 70.3 ($\underline{C}$HOC), 70.6 ($\underline{C}$HOC), 74.8 ($\underline{C}$HOC), 76.4 ($\underline{C}$HOC), 76.6 ($\underline{C}$HOC), 76.8 ($\underline{C}$HOC), 77.0 ($\underline{C}$HOC), 77.7 ($\underline{C}$HOC), 80.1 ($\underline{C}$HOC), 101.6 ($\underline{C}$H(O)$_2$), 103.2 ($\underline{C}$H(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{41}H_{82}NO_{11}$ [M-I$^-$]: 764.5882. Found: 764.5871.



***N*-butyl,*N*-methyl,*N*-octadecyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-ammonium iodide (221i):** (0.19 g, 66%), yellow powder, **mp** 109 °C. **IR (cm^{-1}) υ_{max}:** 728, 907, 1028 and 1070 (CHOCH), 3355 (OH). **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.80 (3H, t, *J*=6.8 Hz, $CH_2C\underline{H}_3$), 0.93 (3H, t, *J*=7.3 Hz, $CH_2C\underline{H}_3$), 1.16 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.19-1.44 (41H, m, 2x$C\underline{H}_2CH_3$, 18x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.47-1.55 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.57-1.67 (6H, m, 3x$C\underline{H}_2CH_2N$), 2.93 (3H, s, $C\underline{H}_3N$), 3.10-3.22 (11H, m, 3x$CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.29 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $C\underline{H}OC$), 3.35-3.39 (1H, m, $C\underline{H}OC$), 3.46 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, $C\underline{H}OC$), 3.54-3.58 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.70-3.78 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.35 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.57 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.6 ($CH_2\underline{C}H_3$), 13.0 ($CH_2\underline{C}H_3$), 19.3 ($C\underline{H}_2CH_3$), 20.6 ($CH\underline{C}H_3$), 21.8 ($\underline{C}H_2CH_2N$), 21.9 ($\underline{C}H_2CH_2N$), 22.3 ($\underline{C}H_2CH_3$), 23.8 ($\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.0 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 28.8 ($\underline{C}H_2(CH_2)_2$), 29.0 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.4 (9x$\underline{C}H_2(CH_2)_2$), 31.7 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 47.4 ($\underline{C}H_3N$), 61.1, 61.4, 61.5, 61.7 (2x$\underline{C}H_2OH$, 3x$CH_2\underline{C}H_2N$), 70.2 ($\underline{C}HOC$), 70.5 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 76.4 ($\underline{C}HOC$), 76.5 ($\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.0 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 80.2 ($\underline{C}HOC$), 101.6 ($\underline{C}H(O)_2$), 103.2 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{44}H_{88}NO_{11}$ [M-I^-]: 806.6352. Found: 806.6346.

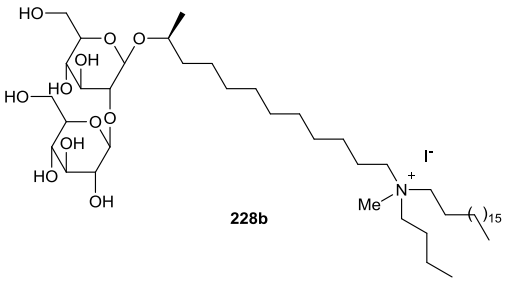


221i

***N*,*N*-dimethyl,*N*-octadecyl-(*(S)*-11-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])dodecan-1-ammonium iodide (228a):** (0.17 g, 70%), white powder.**^{1}H-NMR (400 MHz, MeOD):** δ_H 0.94 (3H, t, *J*=6.6 Hz, $CH_2C\underline{H}_3$), 1.29 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.31-1.57 (45H, m, $C\underline{H}_2CH_3$, 21x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.60-1.68 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.76-1.85 (4H, m, 2x$C\underline{H}_2CH_2N$), 3.13 (6H, s, 2x$C\underline{H}_3N$), 3.25-3.38 (9H, m, 2x$CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.42 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $C\underline{H}OC$), 3.49 (1H, dxd, *J*=8.5 Hz, *J*=8.5 Hz, $C\underline{H}OC$), 3.60 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, $C\underline{H}OC$), 3.67-3.72 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.83-3.91 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.50 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.69 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 13.1 ($CH_2\underline{C}H_3$), 20.6 ($CH\underline{C}H_3$), 22.2 (2x$\underline{C}H_2CH_2N$), 22.3 ($C\underline{H}_2CH_3$), 24.8 ($\underline{C}H_2(CH_2)_2$), 26.0 (2x$\underline{C}H_2(CH_2)_2$), 28.8 (2x$\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.4 (11x$\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 31.7 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 49.9 (2x$\underline{C}H_3N$), 61.4 ($\underline{C}H_2OH$), 61.7 ($\underline{C}H_2OH$), 64.0 (2x$CH_2\underline{C}H_2N$), 70.1 ($\underline{C}HOC$), 70.5 ($\underline{C}HOC$), 74.5 ($\underline{C}HOC$), 76.4 (2x$\underline{C}HOC$), 76.9 (2x$\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 80.4 ($\underline{C}HOC$), 101.5 ($\underline{C}H(O)_2$), 103.3 ($\underline{C}H(O)_2$).

228a

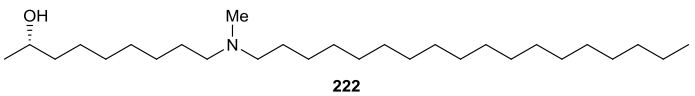
***N*-butyl,*N*-methyl,*N*-octadecyl-(*(S)*-11-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])dodecan-1-ammonium iodide (228b):** (0.05 g, 19%), white powder. **^{1}H-NMR (400 MHz, MeOD):**



228b

δ_H 0.95 (3H, t, *J*=6.8 Hz, $CH_2C\underline{H}_3$), 1.07 (3H, t, *J*=7.3 Hz, $CH_2C\underline{H}_3$), 1.30 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.32-1.56 (47H, m, 2x$C\underline{H}_2CH_3$, 21x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.60-1.69 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.71-1.83 (6H, m, 3x$C\underline{H}_2CH_2N$), 3.09 (3H, s, $C\underline{H}_3N$), 3.26-3.39 (11H, m, 3x$CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.43 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, $C\underline{H}OC$), 3.50 (1H, dxd, *J*=8.4 Hz, *J*=8.4 Hz, $C\underline{H}OC$), 3.60 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, $C\underline{H}OC$), 3.67-3.73 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.83-3.92 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.50 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.69 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.7 ($CH_2\underline{C}H_3$), 13.1 ($CH_2\underline{C}H_3$), 19.4 ($C\underline{H}_2CH_3$), 20.6 ($CH\underline{C}H_3$), 21.9 (2x$C\underline{H}_2CH_2N$), 22.4 ($CH_2\underline{C}H_3$), 23.9 ($\underline{C}H_2CH_2N$), 24.9 ($\underline{C}H_2(CH_2)_2$), 26.0 (2x$C\underline{H}_2(CH_2)_2$), 28.8 (2x$\underline{C}H_2(CH_2)_2$), 29.1-29.6 (15x$C\underline{H}_2(CH_2)_2$), 31.7 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 47.5 ($\underline{C}H_3N$), 61.2-61.7 (2x$C\underline{H}_2OH$, 3x$CH_2C\underline{H}_2N$), 70.1 ($\underline{C}HOC$), 70.5 ($\underline{C}HOC$), 74.5 ($\underline{C}HOC$), 76.4 (3x$\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 80.5 ($\underline{C}HOC$), 101.4 ($\underline{C}H(O)_2$), 103.3 ($\underline{C}H(O)_2$).

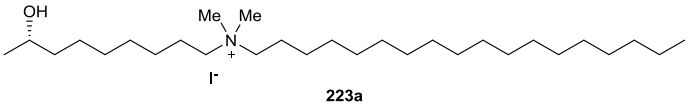
General procedure for the synthesis of *(S)*-9-(methyl(octadecyl)amino)nonan-2-ol (222): *N*-methyl,*N*-octadecyl sophorolipid amine **213h** (0.76 g, 0.73 mmol) was dissolved in 20 mL methanol, a few drops of concentrated sulfuric acid were added, and the mixture was refluxed overnight. Subsequently, an aqueous 2 N sodium hydroxide solution was added until an alkaline pH was obtained. The organic solvent was evaporated under reduced pressure and the resulting water layer was extracted with diethyl ether. The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Compound **222** was purified *via* automated flash chromatography with a hexane/ethyl acetate/thriethylamine mixture as eluent. Purification gradient: 2 CV 5% mixture A, 30 CV 5-60% mixture A, 2 CV 60% mixture A (mixture A = 16% triethylamine in ethyl acetate). Compound **222** was isolated as a viscous colorless oil (0.13 g, 43%).



222

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.88 (3H, t, *J*=6.9 Hz, $C\underline{H}_3CH_2$), 1.18 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.25-1.35 (38H, m, $CH_3C\underline{H}_2$, 18x$C\underline{H}_2(CH_2)_2$), 1.38-1.49 (6H, m, 2x$C\underline{H}_2CH_2N$, $C\underline{H}_2CHCH_3$), 1.94 (1H, br s, O$\underline{H}$), 2.20 (3H, s, CH_3N), 2.28-2.32 (4H, m, 2x$CH_2C\underline{H}_2N$), 3.74-3.82 (1H, m, $C\underline{H}OH$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 14.1 ($\underline{C}H_3CH_2$), 22.7 ($CH_3\underline{C}H_2$), 23.5 ($\underline{C}H_3CH$), 25.7 ($\underline{C}H_2(CH_2)_2$), 27.2 (2x$\underline{C}H_2CH_2N$), 27.6 ($\underline{C}H_2(CH_2)_2$), 27.7 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.6-29.7 (13x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 39.4 ($\underline{C}H_2CHCH_3$), 41.3 ($\underline{C}H_3N$), 57.9 ($\underline{C}H_2N$), 57.9 ($\underline{C}H_2N$), 68.1 ($\underline{C}HOH$). **MS (ESI): m/z** Exact mass calculated for $C_{28}H_{60}NO$ [M+H$^+$]: 426.4669. Found: 426.4655.

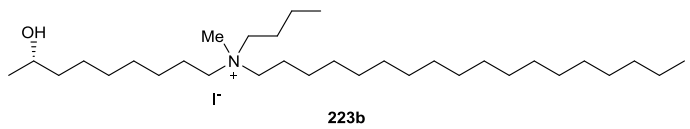
General procedure for synthesis of *N*-(*(S)*-8-hydroxynonyl)-*N*,*N*-dimethyloctadecan-1-ammonium iodide (223a). In a 10 mL pressure resistant vial, 0.071 g of compound **222** (0.17 mmol) was dissolved in a 1:1 mixture of acetone and acetonitrile. The solution was cooled down to 0 °C and 0.05 mL of methyl iodide (0.83 mmol, 5 eq) was added. The vial was closed and heated to 80 °C for 72 hours. The reaction mixture was concentrated under reduced pressure to yield compound **223a** as a yellow waxy solid (0.097 g, 98%).



223a

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.88 (3H, t, *J*=6.8 Hz, $C\underline{H}_3CH_2$), 1.19 (3H, d, *J*=6.2 Hz, $C\underline{H}_3CH$), 1.26-1.46 (40H, m, $C\underline{H}_2CHCH_3$, $CH_3C\underline{H}_2$, 18x$C\underline{H}_2(CH_2)_2$), 1.68-1.77 (4H, m, 2x$C\underline{H}_2CH_2N$), 3.38 (6H, s, 2x$C\underline{H}_3N$), 3.46-3.52 (2H, m, $C\underline{H}_2N$), 3.53-3.58 (2H, m, $C\underline{H}_2N$), 3.67 (1H, s, CHO$\underline{H}$), 3.77-3.84 (1H, m, C$\underline{H}$OH). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 14.1 ($\underline{C}H_3CH_2$), 22.7 (2x$\underline{C}H_2CH_2N$), 22.8 ($CH_3\underline{C}H_2$), 23.6 ($\underline{C}H_3CH$), 25.4 ($\underline{C}H_2(CH_2)_2$), 25.9 ($\underline{C}H_2(CH_2)_2$), 26.2 ($\underline{C}H_2(CH_2)_2$), 28.9 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 29.6 ($\underline{C}H_2(CH_2)_2$), 29.7 (7x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 39.0 ($\underline{C}H_2CHCH_3$), 51.5 (2x$\underline{C}H_3N$), 64.4 (2x$\underline{C}H_2N$), 67.9 ($\underline{C}$HOH). **MS (ESI): m/z** Exact mass calculated for $C_{29}H_{62}NO$ [M-I^-]: 440.4826. Found: 440.4843. **Optical rotation:** $[\alpha]_D^{25}$ 19.0 ± 3.5 (*c* 0.100, EtOH).

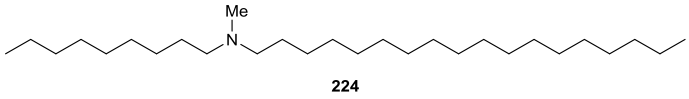
General procedure for synthesis of *N*-butyl,*N*-(*(S)*-8-hydroxynonyl)-*N*-methyloctadecan-1-ammonium iodide (223b): In a 10 mL pressure resistant vial, 0.063 g of compound **222** (0.15 mmol) was dissolved in a 1:1 mixture of acetone and acetonitrile. The solution was cooled down to 0 °C and 0.08 mL of butyl iodide (0.74 mmol, 5 eq) was added. The vial was closed and heated to 80 °C for 72 hours. The reaction mixture was concentrated under reduced pressure to yield compound **223b** as a yellow waxy solid (0.099 g, 87%).



223b

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.88 (3H, t, *J*=6.8 Hz, $C\underline{H}_3CH_2$), 1.02 (3H, t, J=7.3 Hz, $C\underline{H}_3CH_2$), 1.19 (3H, d, J=6.2 Hz, $C\underline{H}_3CH$), 1.26-1.50 (42H, m, $C\underline{H}_2CHCH_3$, 2x$CH_3C\underline{H}_2$, 18x$C\underline{H}_2(CH_2)_2$), 1.65-1.74 (6H, m, 3x$C\underline{H}_2CH_2N$), 3.31 (3H, s, $C\underline{H}_3N$), 3.40-3.49 (6H, m, 3x$C\underline{H}_2N$), 3.67 (1H, s, CHO$\underline{H}$), 3.77-3.84 (1H, m, C$\underline{H}$OH). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 13.8 ($\underline{C}H_3CH_2$), 14.1 ($\underline{C}H_3CH_2$), 19.7 ($CH_3\underline{C}H_2$), 22.5 ($\underline{C}H_2CH_2N$), 22.5 ($\underline{C}H_2CH_2N$), 22.7 ($CH_3\underline{C}H_2$), 23.6 ($\underline{C}H_3CH$), 24.4 ($\underline{C}H_2CH_2N$), 25.4 ($\underline{C}H_2(CH_2)_2$), 26.1 ($\underline{C}H_2(CH_2)_2$), 26.3 ($\underline{C}H_2(CH_2)_2$), 29.0 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 29.6 ($\underline{C}H_2(CH_2)_2$), 29.7 (7x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 39.0 ($\underline{C}H_2CHCH_3$), 49.2 ($\underline{C}H_3N$), 61.6 ($\underline{C}H_2N$), 61.7 ($\underline{C}H_2N$), 61.9 ($\underline{C}H_2N$), 67.9 ($\underline{C}$HOH). **MS (ESI): m/z** Exact mass calculated for $C_{32}H_{68}INO$ [M-I^-]: 482.5295. Found: 482.5309. **Optical rotation:** $[\alpha]_D^{25}$ 9.5 ± 0.5 (*c* 0.105, EtOH).

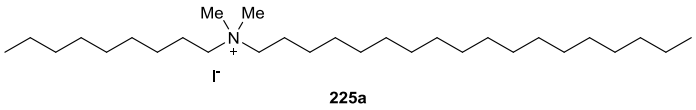
General procedure for synthesis of *N*-methyl,*N*-nonyloctadecan-1-amine (224): Nonanal (0.21 g, 1.44 mmol) and *N*-methyl,*N*-octadecylamine (0.41 g, 1.44 mmol) were dissolved in 10 mL methanol, 0.18 g $NaBH_3CN$ (2.88 mmol, 2 eq) and 0.41 mL acetic acid (7.21 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Compound **224** was isolated as viscous colorless oil without further purification (0.57 g, 97%).



224

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.88 (6H, t, *J*=6.8 Hz, 2x$C\underline{H}_3CH_2$), 1.25-1.32 (42H, m, 2x$CH_3C\underline{H}_2$, 19x$C\underline{H}_2(CH_2)_2$), 1.44-1.50 (4H, m, 2x$C\underline{H}_2CH_2N$), 2.23 (3H, s, $C\underline{H}_3N$), 2.32-2.36 (4H, m, 2xCH_2N). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 14.1 (2x$\underline{C}H_3CH_2$), 22.7 ($\underline{C}H_2CH_2N$), 22.7 ($\underline{C}H_2CH_2N$), 27.1 (2x$\underline{C}H_2CH_2N$), 27.6 (2x$\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.6-29.7 (13x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 42.2 ($\underline{C}H_3N$), 57.8 (2x$\underline{C}H_2N$). **MS (ESI): m/z** Exact mass calculated for $C_{28}H_{60}N$ [M+H$^+$]: 410.4720. Found: 410.4710.

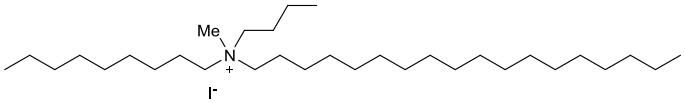
General procedure for synthesis of *N,N*-dimethyl,*N*-nonyloctadecan-1-ammonium iodide (225a): In a 10 mL pressure resistant vial, 0.29 g of compound **224** (0.70 mmol) was dissolved in a 1:1 mixture of acetone and acetonitrile. The solution was cooled down to 0 °C and 0.22 mL of methyl iodide (3.49 mmol, 5 eq) was added. The vial was closed and heated to 80 °C for 72 hours. The reaction mixture was concentrated under reduced pressure to yield compound **225a** as an orange waxy solid (0.39 g, quant.).



225a

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.81 (6H, t, *J*=6.7 Hz, 2xCH_3CH_2), 1.19-1.36 (42H, m, 2x$CH_3C\underline{H}_2$, 19x$C\underline{H}_2(CH_2)_2$), 1.62-1.70 (4H, m, 2x$C\underline{H}_2CH_2N$), 3.32 (6H, s, 2x$C\underline{H}_3N$), 3.44-3.48 (4H, m, 2xCH_2N). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 14.1 ($\underline{C}H_3CH_2$), 14.1 ($\underline{C}H_3CH_2$), 22.6 ($CH_3\underline{C}H_2$), 22.7 ($CH_3\underline{C}H_2$), 22.8 (2x$\underline{C}H_2CH_2N$), 26.2 (2x$\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 29.6 ($\underline{C}H_2(CH_2)_2$), 29.6-29.7 (7x$\underline{C}H_2(CH_2)_2$), 31.8 ($\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 51.6 (2x$\underline{C}H_3N$), 64.2 (2x$\underline{C}H_2N$). **MS (ESI): m/z** Exact mass calculated for $C_{29}H_{62}N$ [M-I$^-$]: 424.4877. Found: 424.4881.

General procedure for synthesis of *N*-butyl,*N*-methyl,*N*-nonyloctadecan-1-ammonium iodide (225b): In a 10 mL pressure resistant vial, 0.28 g of compound **221** (0.70 mmol) was dissolved in a 1:1 mixture of acetone and acetonitrile. The solution was cooled down to 0 °C and 0.39 mL of butyl iodide (3.47 mmol, 5 eq) was added. The vial was closed and heated to 80 °C for 72 hours. The reaction mixture was concentrated under reduced pressure to yield compound **222b** as a yellow waxy solid (0.40 g, 97%).



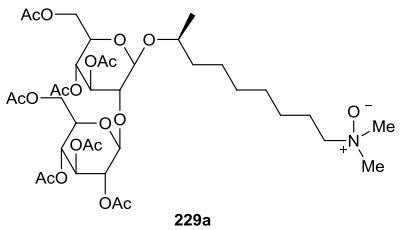
225b

^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.88 (6H, t, *J*=6.5 Hz, 2xCH_3CH_2), 1.02 (3H, t, *J*=7.3 Hz, CH_3CH_2), 1.26-1.50 (44H, m, 3xCH_3CH_2, 19x$CH_2(CH_2)_2$), 1.67-1.76 (6H, m, 3xCH_2CH_2N), 3.29 (3H, s, CH_3N), 3.42-3.50 (6H, m, 3xCH_2N). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 13.8 (CH_3CH_2), 14.1 (CH_3CH_2), 14.1 (CH_3CH_2), 19.7 (CH_3CH_2), 22.5 (2xCH_2CH_2N), 22.6 (CH_3CH_2), 22.7 (CH_3CH_2), 24.5 (CH_2CH_2N), 26.3 (2x$CH_2(CH_2)_2$), 29.1 ($CH_2(CH_2)_2$), 29.2 ($CH_2(CH_2)_2$), 29.2 ($CH_2(CH_2)_2$), 29.3 ($CH_2(CH_2)_2$), 29.3 ($CH_2(CH_2)_2$), 29.4 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 29.6 ($CH_2(CH_2)_2$), 29.6-29.7 (7x$CH_2(CH_2)_2$), 31.8 ($CH_2(CH_2)_2$), 31.9 ($CH_2(CH_2)_2$), 49.3 (CH_3N), 61.7 (CH_2N), 61.8 (2xCH_2N). **MS (ESI): m/z** Exact mass calculated for $C_{32}H_{68}N$ [M-I$^-$]: 466.5346. Found: 466.5356.

4.2.4. Synthesis of sophorolipid amine oxides

General procedure for synthesis of peracetylated sophorolipid amine oxides (229): In a 50 mL flask, sophorolipid amine **213** (0.41 mmol, 1 eq) was dissolved in dry THF, cooled to 0 °C and 0.1 g *m*CPBA (0.58 mmol, 1.4 eq) was added. The mixture was stirred for 30 minutes at 0 °C and subsequently for 1.5 h at room temperature. The mixture was concentrated under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed three times with a saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated under reduced pressure to yield pure amine oxide **229** as a viscous colorless oil.

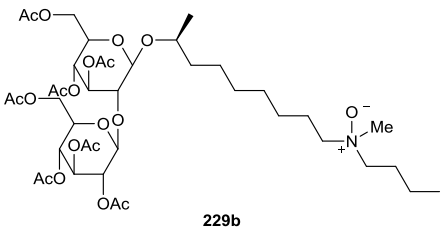
***N*,*N*-dimethyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229a):** (0.36 g, 79%), **IR (cm^{-1}) υ_{max}:** 1036 and 1064 (CHOCH), 1221 (COAc), 1367, 1746 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ_H 1.22 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.26-1.44 (9H, m, 4x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.53-1.61 (1H, m, $CH_aH_bCHCH_3$), 1.89-1.96 (2H, m, CH_2CH_2N), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.02 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.09 (3H, s, CH_3C=O), 3.21 (6H, s, 2xCH_3N), 3.27-3.31 (2H, m, CH_2CH_2N), 3.63-3.74 (4H, m, 2x$CHCH_2OAc$, CH_3CHO, CHOC), 4.06-4.11 (2H, m, 2x$CHCH_aH_bOAc$), 4.23-4.29 (2H, m, 2x$CHCH_aH_bOAc$), 4.46 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.70 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.89 (1H, dxd, *J*=9.5 Hz,



229a

J=8.1 Hz, CHOC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.03 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.12 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 20.4 (CH_3C=O), 20.6 (3xCH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.4 ($CHCH_3$), 23.9 (CH_2CH_2N), 24.8 ($CH_2(CH_2)_2$), 26.7 ($CH_2(CH_2)_2$), 29.4 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 36.5 (CH_2CHCH_3), 58.2 (2xCH_3N), 62.2 (CH_2OAc), 62.2 (CH_2OAc), 68.4 (CHOC), 68.8 (CHOC), 71.3 (CHOC), 71.5 (CHOC), 71.6 (CH_2CH_2N), 71.7 (CHOC), 73.0 (CHOC), 74.8 (CHOC), 77.7 (CHOC), 77.9 (CHOC), 100.5 ($CH(O)_2$), 101.2 ($CH(O)_2$), 169.5 (CH_3C=O), 169.5 (CH_3C=O), 169.8 (CH_3C=O), 170.0 (CH_3C=O), 170.2 (CH_3C=O), 170.6 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{37}H_{60}NO_{19}$ [M+H$^+$]: 822.3754. Found: 822.3759.

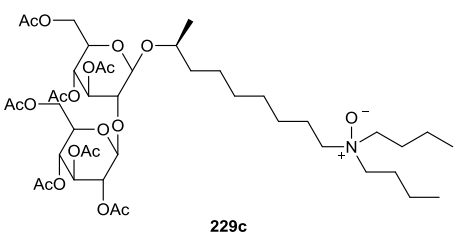
***N*-butyl,*N*-methyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229b):** (0.29 g, 87%), **IR (cm^{-1}) υ_{max}:** 1036 and 1064 (CHOCH), 1225 (COAc), 1367, 1746 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ_H 0.91 (3H, t, *J*=7.3 Hz, CH_2CH_3), 1.15 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.19-1.36 (11H, m, 5x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.46-1.54 (1H, m, $CH_aH_bCHCH_3$), 1.72-1.83 (4H, m, 2xCH_2CH_2N), 1.92 (3H, s, CH_3C=O), 1.93 (3H, s, CH_3C=O), 1.95 (3H, s, CH_3C=O), 1.96 (3H, s, CH_3C=O), 1.99 (3H, s, CH_3C=O), 2.01 (3H, s, CH_3C=O), 2.01 (3H, s, CH_3C=O), 3.01 (3H, s, CH_3N), 3.10-3.17 (4H, m, 2xCH_2CH_2N), 3.56-3.69 (4H, m, 2xCHCH$_2$OAc, CH_3CHO, CHOC), 3.99-4.04 (2H, m, 2x$CHCH_aH_bOAc$), 4.16-4.22 (2H, m, 2x$CHCH_aH_bOAc$), 4.39 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.64 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.82 (1H, dxd, *J*=9.4 Hz, *J*=8.2 Hz, CHOC), 4.86 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 4.97 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.06 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.10 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC).



229b

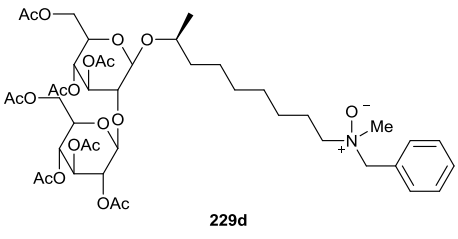
^{13}C-NMR (100MHz, $CDCl_3$): δ_C 13.8 (CH_2CH_3), 20.1 (CH_2CH_3), 20.4 (CH_3C=O), 20.5 (2xCH_3C=O), 20.5 (CH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.3 ($CHCH_3$), 23.6 (CH_2CH_2N), 24.8 ($CH_2(CH_2)_2$), 25.4 (CH_2CH_2N), 26.8 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 36.4 (CH_2CHCH_3), 55.7 (CH_3N), 62.1 (CH_2OAc), 62.2 (CH_2OAc), 68.3 (CHOC), 68.7 (CH_2CH_2N), 68.8 (CHOC), 69.1 (CH_2CH_2N), 71.2 (CHOC), 71.5 (CHOC), 71.7 (CHOC), 73.0 (CHOC), 74.7 (CHOC), 77.6 (CHOC), 77.9 (CHOC), 100.4 ($CH(O)_2$), 101.2 ($CH(O)_2$), 169.4 (CH_3C=O), 169.4 (CH_3C=O), 169.7 (CH_3C=O), 170.0 (CH_3C=O), 170.1 (CH_3C=O), 170.5 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{40}H_{66}NO_{19}$ [M+H$^+$]: 864.4224. Found: 864.4260.

***N,N*-dibutyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229c):** (0.60 g, 93%), **IR (cm^{-1}) υ_{max}:** 1034 and 1063 (CHOCH), 1217 (COAc), 1367, 1745 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ_H 0.97 (6H, t, *J*=7.3 Hz, 2x$CH_2C\underline{H}_3$), 1.21 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.29-1.42 (13H, m, 6x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.53-1.61 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.73-1.84 (6H, m, 3x$C\underline{H}_2CH_2N$), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.00 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.03 (3H, s, $C\underline{H}_3C$=O), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.08 (3H, s, $C\underline{H}_3C$=O), 2.08 (3H, s, $C\underline{H}_3C$=O), 3.09-3.13 (6H, m, 3x$CH_2C\underline{H}_2N$), 3.63-3.75 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.06-4.10 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.23-4.29 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.47 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.89 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, $C\underline{H}OC$), 5.04 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, $C\underline{H}OC$), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$), 5.16 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 13.9 (2x$CH_2\underline{C}H_3$), 20.2 (2x$\underline{C}H_2CH_3$), 20.4 ($\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 (2x$\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.3 ($CH\underline{C}H_3$), 23.2 ($\underline{C}H_2CH_2N$), 24.9 ($\underline{C}H_2(CH_2)_2$), 25.1 (2x$\underline{C}H_2CH_2N$), 27.0 ($\underline{C}H_2(CH_2)_2$), 29.5 (2x$\underline{C}H_2(CH_2)_2$), 36.4 ($\underline{C}H_2CHCH_3$), 62.1 ($\underline{C}H_2OAc$), 62.2 ($\underline{C}H_2OAc$), 65.9 (2x$CH_2\underline{C}H_2N$), 66.3 ($CH_2\underline{C}H_2N$), 68.3 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 71.3 ($\underline{C}HOC$), 71.6 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 77.9 ($\underline{C}HOC$), 100.4 ($\underline{C}H(O)_2$), 101.1 ($\underline{C}H(O)_2$), 169.3 ($CH_3\underline{C}$=O), 169.4 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.2 ($CH_3\underline{C}$=O), 170.5 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{43}H_{72}NO_{19}$ [M+H$^+$]: 906.4693. Found: 906.4711.



229c

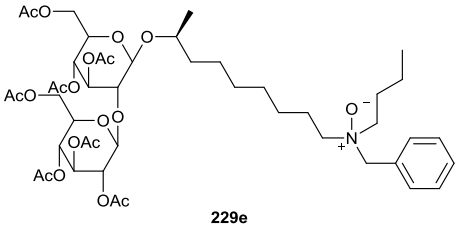
***N*-benzyl,*N*,methyl-(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229d):** (0.27 g, 95%), **IR (cm^{-1}) υ_{max}:** 1033 and 1062 (CHOCH), 1215 (COAc), 1366, 1743 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ_H 1.22 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.26-1.43 (9H, m, 4x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.53-1.61 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.88-1.94 (1H, m, $C\underline{H}_aH_bCH_2N$), 1.97 (3H, s, $C\underline{H}_3C$=O), 2.00 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.02 (3H, s, $C\underline{H}_3C$=O), 2.04-2.09 (1H, m, $CH_a\underline{H}_bCH_2N$), 2.06 (3H, s, $C\underline{H}_3C$=O), 2.07 (3H, s, $C\underline{H}_3C$=O), 2.08 (3H, s, $C\underline{H}_3C$=O), 3.02 (3H, s, $C\underline{H}_3N$), 3.14-3.18 (2H, m, $CH_2C\underline{H}_2N$), 3.63-3.75 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 4.06-4.11 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.23-4.29 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.38 (1H, d, *J*=12.9 Hz, $C_{arom}C\underline{H}_aH_bN$), 4.44 (1H, d, *J*=12.9 Hz, $C_{arom}CH_a\underline{H}_bN$), 4.46-4.48 (1H, m, $C\underline{H}(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.87-4.91 (1H, m, $C\underline{H}OC$), 4.93 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, $C\underline{H}OC$), 5.04 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, $C\underline{H}OC$), 5.13 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$), 5.14-5.20 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, $C\underline{H}OC$), 7.40-7.44 (3H, m, 3x$C\underline{H}_{arom}$), 7.53-7.55 (2H, m, 2x$C\underline{H}_{arom}$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 20.4 ($\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 21.3 ($CH\underline{C}H_3$), 23.2



229d

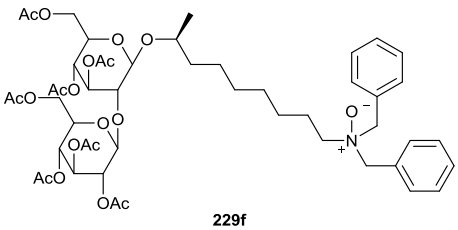
($\underline{C}H_2CH_2N$), 24.8 ($\underline{C}H_2(CH_2)_2$), 26.8 ($\underline{C}H_2(CH_2)_2$), 29.4 ($\underline{C}H_2(CH_2)_2$), 29.6 ($\underline{C}H_2(CH_2)_2$), 36.4 ($\underline{C}H_2CHCH_3$), 55.0 ($\underline{C}H_3N$), 62.1 ($\underline{C}H_2OAc$), 62.2 ($\underline{C}H_2OAc$), 68.3 ($\underline{C}HOC$), 68.4 ($CH_2\underline{C}H_2N$), 68.8 ($\underline{C}HOC$), 71.3 ($\underline{C}HOC$), 71.5 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.1 ($C_{arom}\underline{C}H_2N$), 74.7 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 77.8 ($\underline{C}HOC$), 100.4 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 128.7 (2x$\underline{C}H_{arom}$), 129.5 ($\underline{C}H_{arom}$), 130.7 ($\underline{C}_{arom}$), 132.2 (2x$\underline{C}H_{arom}$), 169.4 ($CH_3\underline{C}$=O), 169.4 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.2 ($CH_3\underline{C}$=O), 170.5 ($CH_3\underline{C}$=O), 170.6 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{43}H_{64}NO_{19}$ [M+H$^+$]: 898.4067. Found: 898.4073.

***N*-benzyl,*N*-butyl-((*S*)-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229e):** (0.32 g, 94%), **IR (cm^{-1}) υ_{max}:** 1037 and 1065 (CHOCH), 1227 (COAc), 1367, 1749 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$):** δ_H 0.89 (3H, t, *J*=7.4 Hz, $CH_2C\underline{H}_3$), 1.15 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.19-1.34 (11H, m, 5x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.46-1.54 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.70-1.80 (2H, m, 2x$C\underline{H}_aH_bCH_2N$), 1.83-1.91 (2H, m, 2x$CH_a\underline{H}_bCH_2N$), 1.90 (3H, s, $C\underline{H}_3C$=O), 1.93 (3H, s, $C\underline{H}_3C$=O), 1.94 (3H, s, $C\underline{H}_3C$=O), 1.96 (3H, s, $C\underline{H}_3C$=O), 1.99 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.01 (3H, s, $C\underline{H}_3C$=O), 2.99-3.05 (4H, m, 2x$CH_2C\underline{H}_2N$), 3.56-3.68 (4H, m, 2x$C\underline{H}CH_2OAc$, $CH_3C\underline{H}O$, $C\underline{H}OC$), 3.99-4.04 (2H, m, 2x$CHC\underline{H}_aH_bOAc$), 4.16-4.22 (2H, m, 2x$CHCH_a\underline{H}_bOAc$), 4.29 (2H, s, $C_{arom}C\underline{H}_2N$), 4.40 (1H, d, *J*=7.6 Hz, $C\underline{H}(O)_2$), 4.64 (1H, d, *J*=8.0 Hz, $C\underline{H}(O)_2$), 4.81-4.85 (1H, m, $C\underline{H}OC$), 4.86 (1H, dxd, *J*=9.8 Hz, *J*=9.8 Hz, $C\underline{H}OC$), 4.98 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, $C\underline{H}OC$), 5.06 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, $C\underline{H}OC$), 5.07-5.13 (1H, m, $C\underline{H}OC$), 7.31-7.35 (3H, m, 3x$C\underline{H}_{arom}$), 7.49-7.51 (2H, m, 2x$C\underline{H}_{arom}$). **^{13}C-NMR (100MHz, $CDCl_3$):** δ_C 13.7 ($CH_2\underline{C}H_3$), 20.1 ($\underline{C}H_2CH_3$), 20.3 ($\underline{C}H_3C$=O), 20.4 (2x$\underline{C}H_3C$=O), 20.4 ($\underline{C}H_3C$=O), 20.5 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.7 ($\underline{C}H_3C$=O), 21.2 ($CH\underline{C}H_3$), 23.3 ($\underline{C}H_2CH_2N$), 24.8 ($\underline{C}H_2(CH_2)_2$), 25.2 ($\underline{C}H_2CH_2N$), 26.9 ($\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 29.5 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 62.2 ($CH_2\underline{C}H_2N$), 62.4 ($CH_2\underline{C}H_2N$), 65.1 ($CH_2\underline{C}H_2N$), 65.5 ($CH_2\underline{C}H_2N$), 68.7 ($\underline{C}HOC$), 69.1 ($\underline{C}HOC$), 70.6 ($C_{arom}\underline{C}H_2N$), 71.4 ($\underline{C}HOC$), 71.7 ($\underline{C}HOC$), 71.9 ($\underline{C}HOC$), 73.1 ($\underline{C}HOC$), 74.8 ($\underline{C}HOC$), 77.7 ($\underline{C}HOC$), 77.8 ($\underline{C}HOC$), 100.5 ($\underline{C}H(O)_2$), 101.1 ($\underline{C}H(O)_2$), 128.4 (2x$\underline{C}H_{arom}$), 129.2 ($\underline{C}H_{arom}$), 130.7 ($\underline{C}_{arom}$), 132.1 (2x$\underline{C}H_{arom}$), 169.2 ($CH_3\underline{C}$=O), 169.3 ($CH_3\underline{C}$=O), 169.6 ($CH_3\underline{C}$=O), 169.8 ($CH_3\underline{C}$=O), 170.0 ($CH_3\underline{C}$=O), 170.3 ($CH_3\underline{C}$=O), 170.3 ($CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{46}H_{70}NO_{19}$ [M+H$^+$]: 940.4537. Found: 940.4575.



229e

***N,N*-dibenzyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229f):** (0.27 g, 63%), **IR (cm^{-1}) υ_{max}:** 1035 and 1064 (CHOCH), 1223 (COAc), 1367, 1747 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$): δ_H** 1.09-1.33 (9H, m, 4x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.14 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.46-1.52 (1H, m, $CH_aH_bCHCH_3$), 1.85-1.94 (2H, m, CH_2CH_2N), 1.89 (3H, s, CH_3C=O), 1.93 (3H, s, CH_3C=O), 1.94 (3H, s, CH_3C=O), 1.96 (3H, s, CH_3C=O), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.01 (3H, s, CH_3C=O), 2.78-2.82 (2H, m, CH_2CH_2N), 3.56-3.65 (4H, m, 2x$CHCH_2OAc$, CH_3CHO, CHOC), 4.00-4.03 (2H, m, 2x$CHCH_aH_bOAc$), 4.16-4.22 (2H, m, 2x$CHCH_aH_bOAc$), 4.25 (2H, d, *J*=12.5 Hz, 2x$C_{arom}CH_aH_bN$), 4.34 (2H, dxd, *J*=12.5 Hz, *J*=3.7 Hz, 2x$C_{arom}CH_aH_bN$), 4.39 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.64 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.84 (1H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, CHOC), 4.86 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 4.98 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.06 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.10 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC), 7.31-7.35 (6H, m, 6xCH_{arom}), 7.52-7.57 (4H, m, 4xCH_{arom}). **^{13}C-NMR (100MHz, $CDCl_3$): δ_C** 20.5 (CH_3C=O), 20.6 (2xCH_3C=O), 20.6 (CH_3C=O), 20.7 (CH_3C=O), 20.7 (CH_3C=O), 20.8 (CH_3C=O), 21.3 ($CHCH_3$), 23.7 (CH_2CH_2N), 24.8 ($CH_2(CH_2)_2$), 26.7 ($CH_2(CH_2)_2$), 29.4 ($CH_2(CH_2)_2$), 29.5 ($CH_2(CH_2)_2$), 36.4 (CH_2CHCH_3), 62.1 (CH_2CH_2N), 62.3 (CH_2CH_2N),63.7 (CH_2CH_2N), 68.4 (CHOC), 68.9 (CHOC), 70.9 (2x$C_{arom}CH_2N$), 71.3 (CHOC), 71.6 (CHOC), 17.8 (CHOC), 73.0 (CHOC), 74.7 (CHOC), 77.7 (CHOC), 77.8 (CHOC), 100.5 ($CH(O)_2$), 101.1 ($CH(O)_2$), 128.5 (4xCH_{arom}), 129.3 (2xCH_{arom}), 130.8 (2xC_{arom}), 132.3 (4xCH_{arom}), 169.4 (CH_3C=O), 169.5 (CH_3C=O), 169.7 (CH_3C=O), 170.0 (CH_3C=O), 170.2 (CH_3C=O), 170.6 (CH_3C=O), 170.6 (CH_3C=O). **MS (ESI): m/z** Exact mass calculated for $C_{49}H_{68}NO_{19}$ $[M+H^+]$: 974.4380. Found: 974.4412.

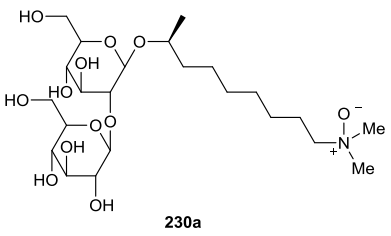


***N*-methyl,*N*-octadecyl-(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (229g):** (0.52 g, 92%), **IR (cm^{-1}) υ_{max}:** 1036 and 1064 (CHOCH), 1223 (COAc), 1366, 1747 (C=O). **^{1}H-NMR (400MHz, $CDCl_3$): δ_H** 0.88 (3H, t, *J*=6.8 Hz, CH_2CH_3), 1.22 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.24-1.42 (39H, m, 19x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.54-1.60 (1H, m, $CH_aH_bCHCH_3$), 1.81-1.89 (4H, m, 4xCH_2CH_2N), 1.99 (3H, s, CH_3C=O), 2.00 (3H, s, CH_3C=O), 2.01 (3H, s, CH_3C=O), 2.03 (3H, s, CH_3C=O), 2.06 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 2.08 (3H, s, CH_3C=O), 3.08 (3H, s, CH_3N), 3.15-3.22 (4H, m, 2xCH_2CH_2N), 3.63-3.74 (4H, m, 2x$CHCH_2OAc$, CH_3CHO, CHOC), 4.06-4.10 (2H, m, 2x$CHCH_aH_bOAc$), 4.23-4.29 (2H, m, 2x$CHCH_aH_bOAc$), 4.46 (1H, d, *J*=7.6 Hz, $CH(O)_2$), 4.71 (1H, d, *J*=8.0 Hz, $CH(O)_2$), 4.89 (1H, dxd, *J*=9.4 Hz, *J*=8.1 Hz, CHOC), 4.93 (1H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, CHOC), 5.04 (1H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, CHOC), 5.12 (1H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, CHOC), 5.17 (1H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, CHOC). **^{13}C-NMR (100MHz,**

$CDCl_3$): δ_C 14.1 ($CH_2\underline{C}H_3$), 20.4 ($\underline{C}H_3C{=}O$), 20.6 (3x$\underline{C}H_3C{=}O$), 20.7 ($\underline{C}H_3C{=}O$), 20.7 ($\underline{C}H_3C{=}O$), 20.8 ($\underline{C}H_3C{=}O$), 21.3 ($CH\underline{C}H_3$), 22.7 ($\underline{C}H_2CH_3$), 23.5 ($\underline{C}H_2CH_2N$), 23.7 ($\underline{C}H_2CH_2N$), 24.8 ($\underline{C}H_2(CH_2)_2$), 26.8 ($\underline{C}H_2(CH_2)_2$), 26.9 ($\underline{C}H_2(CH_2)_2$), 29.3-29.7 (13x$\underline{C}H_2(CH_2)_2$), 31.9 ($\underline{C}H_2(CH_2)_2$), 36.4 ($\underline{C}H_2CHCH_3$), 55.9 ($\underline{C}H_3N$), 62.1 ($CH_2\underline{C}H_2N$), 62.2 ($CH_2\underline{C}H_2N$), 68.4 ($\underline{C}HOC$), 68.8 ($\underline{C}HOC$), 69.2 ($CH_2\underline{C}H_2N$), 69.3 ($CH_2\underline{C}H_2N$), 71.3 ($\underline{C}HOC$), 71.5 ($\underline{C}HOC$), 71.8 ($\underline{C}HOC$), 73.0 ($\underline{C}HOC$), 74.7 ($\underline{C}HOC$), 77.7 ($\underline{C}HOC$), 77.9 ($\underline{C}HOC$), 100.5 ($\underline{C}H(O)_2$), 101.2 ($\underline{C}H(O)_2$), 169.4 ($CH_3\underline{C}{=}O$), 169.5 ($CH_3\underline{C}{=}O$), 169.7 ($CH_3\underline{C}{=}O$), 170.0 ($CH_3\underline{C}{=}O$), 170.2 ($CH_3\underline{C}{=}O$), 170.5 ($CH_3\underline{C}{=}O$), 170.6 ($CH_3\underline{C}{=}O$). **MS (ESI): m/z** Exact mass calculated for $C_{54}H_{94}NO_{19}$ [$M+H^+$]: 1060.6415. Found: 1060.6417.

General procedure for synthesis of sophorolipid amine oxides (230): In a 50 mL flask, sophorolipid amine oxide **229** (0.45 mmol, 1 eq) was dissolved in a methanol/water mixture (1:1) and 13 mL Et_3N (0.90 mmol, 2 eq) was added. The mixture was stirred for 2 h at reflux temperature and concentrated under reduced pressure to yield pure sophorolipid amine oxide **230** as an off-white sticky powder.

***N*,*N*-dimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (230a):** (0.23 g, quant.), **IR (cm^{-1}) υ_{max}:** 1025 and 1071 (CHOCH), 3348 (OH). **^{1}H-NMR (400MHz, MeOD):** δ_H 1.16 (3H, d, *J*=6.2 Hz, $CH\underline{C}H_3$), 1.19-1.44 (9H, m, 4x$\underline{C}H_2(CH_2)_2$, $\underline{C}H_aH_bCHCH_3$), 1.44-1.54 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.72-1.80 (2H, m, 2x$\underline{C}H_2CH_2N$), 3.09 (6H, s, 2x$\underline{C}H_3N$), 3.12-3.24 (7H, m, 5x$\underline{C}HOC$, $CH_2\underline{C}H_2N$), 3.27 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, $\underline{C}HOC$), 3.37 (1H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, $\underline{C}HOC$), 3.46 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $\underline{C}HOC$), 3.52-3.58 (2H, m, 2x$CH\underline{C}H_aH_bOH$), 3.69-3.77 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $\underline{C}HOC$), 4.34 (1H, d, *J*=7.7 Hz, $\underline{C}H(O)_2$), 4.57 (1H, d, *J*=7.8 Hz, $\underline{C}H(O)_2$). **^{13}C-NMR (100MHz, MeOD):** δ_C 20.6 ($CH\underline{C}H_3$), 23.0 ($\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.2 ($\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 56.7 (2x$\underline{C}H_3N$), 61.4 ($\underline{C}H_2OH$), 61.7 ($\underline{C}H_2OH$), 70.1 ($\underline{C}HOC$), 70.3 ($CH_2\underline{C}H_2N$), 70.5 ($\underline{C}HOC$), 74.6 ($\underline{C}HOC$), 76.4 ($\underline{C}HOC$), 76.4 ($\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.0 ($\underline{C}HOC$), 77.7 ($\underline{C}HOC$), 80.0 ($\underline{C}HOC$), 101.6 ($\underline{C}H(O)_2$), 103.0 ($\underline{C}H(O)_2$).**MS (ESI): m/z** Exact mass calculated for $C_{23}H_{46}NO_{12}$ [$M+H^+$]: 528.3015. Found: 528.3012.



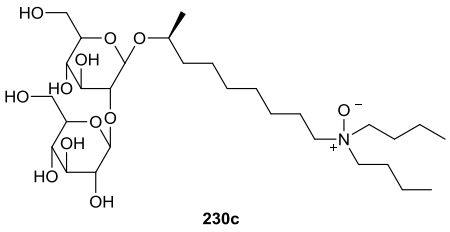
230a

***N*-butyl,*N*-methyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (230b):** (0.11 g, 95%), **IR (cm^{-1}) υ_{max}:** 1032 and 1072 (CHOCH), 3352 (OH). **^{1}H-NMR (400MHz, MeOD):** δ_H 0.91 (3H, t, *J*=7.4 Hz, $CH_2C\underline{H}_3$), 1.15 (3H, d, *J*=6.2 Hz, $CH\underline{C}H_3$), 1.18-1.44 (11H, m, 5x$\underline{C}H_2(CH_2)_2$, $\underline{C}H_aH_bCHCH_3$), 1.44-1.55 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.65-1.77 (4H, m, 2x$\underline{C}H_2CH_2N$), 3.02 (3H, s, $\underline{C}H_3N$), 3.08-3.23 (9H, m, 5x$\underline{C}HOC$, 2x$CH_2\underline{C}H_2N$), 3.27 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, $\underline{C}HOC$), 3.35-3.39 (1H, m, $\underline{C}HOC$), 3.45 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, $\underline{C}HOC$), 3.52-3.58 (2H, m, 2x$CH\underline{C}H_aH_bOH$), 3.69-3.77 (3H, m, 2x$CHCH_a\underline{H}_bOH$,

230b

CHOC), 4.34 (1H, d, *J*=7.7 Hz, CH(O)$_2$), 4.57 (1H, d, *J*=7.8 Hz, CH(O)$_2$). **^{13}C-NMR (100MHz, MeOD): δ_C** 12.7 (CH_2CH_3), 19.5 (CH_2CH_3), 20.6 ($CHCH_3$), 22.7 (CH_2CH_2N), 24.7 (CH_2CH_2N, $CH_2(CH_2)_2$), 26.2 ($CH_2(CH_2)_2$), 29.1 ($CH_2(CH_2)_2$), 29.3 ($CH_2(CH_2)_2$), 36.5 (CH_2CHCH_3), 53.5 (CH_3N), 61.4 (CH_2OH), 61.7 (CH_2OH), 68.0 (CH_2CH_2N), 68.2 (CH_2CH_2N), 70.1 (CHOC), 70.4 (CHOC), 74.6 (CHOC), 76.4 (2xCHOC), 76.9 (CHOC), 77.0 (CHOC), 77.6 (CHOC), 80.1 (CHOC), 101.6 (CH(O)$_2$), 103.1 (CH(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{26}H_{52}NO_{12}$ [M+H$^+$]: 570.3484. Found: 570.3499.

***N,N*-dibutyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide**



230c

(230c): (0.26 g, 84%), **IR (cm^{-1}) υ_{max}:** 1027 and 1073 (CHOCH), 3334 (OH). **^{1}H-NMR (400MHz, MeOD): δ_H** 1.02 (6H, t, *J*=7.4 Hz, 2xCH$_2$CH$_3$), 1.27 (3H, d, *J*=6.2 Hz, CHCH$_3$), 1.31-1.55 (13H, m, 6xCH$_2$(CH$_2$)$_2$, CH$_a$H$_b$CHCH$_3$), 1.58-1.66 (1H, m, CH$_a$H$_b$CHCH$_3$), 1.71-1.82 (6H, m, 3xCH$_2$CH$_2$N), 3.20-3.35 (11H, m, 3xCH$_2$CH$_2$N, 5xCHOC), 3.39 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, CHOC), 3.46-3.50 (1H, m, CHOC), 3.57 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, CHOC), 3.64-3.70 (2H, m, 2xCHCH$_a$H$_b$OH), 3.81-3.89 (3H, m, 2xCHCH$_a$H$_b$OH, CHOC), 4.46 (1H, d, *J*=7.7 Hz, CH(O)$_2$), 4.68 (1H, d, *J*=7.8 Hz, CH(O)$_2$). **^{13}C-NMR (100MHz, MeOD): δ_C** 12.7 (2xCH$_2$CH$_3$), 19.6 (2xCH$_2$CH$_3$), 20.6 (CHCH$_3$), 22.4 (CH$_2$CH$_2$N), 24.5 (2xCH$_2$CH$_2$N), 24.7 (CH$_2$(CH$_2$)$_2$), 26.3 (CH$_2$(CH$_2$)$_2$), 29.1 (CH$_2$(CH$_2$)$_2$), 29.4 (CH$_2$(CH$_2$)$_2$), 36.5 (CH$_2$CHCH$_3$), 61.4 (CH$_2$OH), 61.7 (CH$_2$OH), 64.8 (2xCH$_2$CH$_2$N), 65.1 (CH$_2$CH$_2$N), 70.1 (CHOC), 70.4 (CHOC), 74.6 (CHOC), 76.4 (2xCHOC), 76.9 (CHOC), 76.9 (CHOC), 77.6 (CHOC), 80.2 (CHOC), 101.5 (CH(O)$_2$), 103.1 (CH(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{29}H_{58}NO_{12}$ [M+H$^+$]: 612.3954. Found: 612.3965.

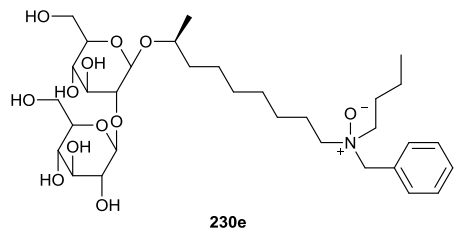
***N,N*-dimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide**

(230d): (0.38 g, quant.), **IR (cm^{-1}) υ_{max}:** 1023 and 1072 (CHOCH), 3332 (OH). **^{1}H-NMR (400MHz, MeOD): δ_H** 1.28 (3H, d, *J*=6.2 Hz, CHCH$_3$), 1.31-1.56 (9H, m, 4xCH$_2$(CH$_2$)$_2$, CH$_a$H$_b$CHCH$_3$), 1.58-1.69 (1H, m, CH$_a$H$_b$CHCH$_3$), 1.86-1.95 (1H, m, CH$_a$H$_b$CHCH$_3$), 1.98-2.06 (1H, m, CH$_a$H$_b$CHCH$_3$), 3.02 (3H, s, CH$_3$N), 3.21-3.37 (7H, m, CH$_2$CH$_2$N, 5xCHOC), 3.40 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, CHOC), 3.47-3.51 (1H, m, CHOC), 3.58 (1H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, CHOC), 3.64-3.70 (2H, m, 2xCHCH$_a$H$_b$OH), 3.81-3.89 (3H, m, 2xCHCH$_a$H$_b$OH, CHOC), 4.43 (1H, d, *J*=12.7 Hz, C$_{arom}$CH$_a$H$_b$N), 4.46 (1H, d, *J*=7.7 Hz, CH(O)$_2$), 4.48 (1H, d, *J*=12.7 Hz, C$_{arom}$CH$_a$H$_b$N), 4.69 (1H, d, *J*=7.8 Hz, CH(O)$_2$), 7.44-7.49 (3H, m, 3xCH$_{arom}$), 7.59-7.62 (2H, m, 2xCH$_{arom}$). **^{13}C-NMR (100MHz, MeOD): δ_C** 20.6 (CHCH$_3$), 22.6 (CH$_2$CH$_2$N), 24.7 (CH$_2$(CH$_2$)$_2$), 26.3 (CH$_2$(CH$_2$)$_2$), 29.1 (CH$_2$(CH$_2$)$_2$), 29.3 (CH$_2$(CH$_2$)$_2$), 36.5 (CH$_2$CHCH$_3$), 52.9 (CH$_3$N), 61.4 (CH$_2$OH), 61.7 (CH$_2$OH), 68.3 (CH$_2$CH$_2$N), 70.1 (CHOC), 70.4 (CHOC), 72.3 (C$_{arom}$CH$_2$N), 74.6 (CHOC), 76.4 (CHOC), 76.4

230d

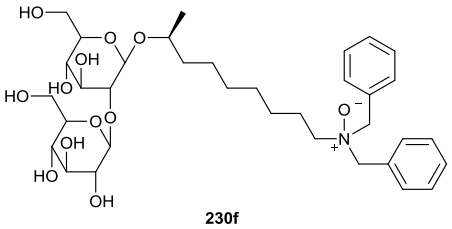
(CHOC), 76.9 (CHOC), 77.0 (CHOC), 77.7 (CHOC), 80.1 (CHOC), 101.6 ($CH(O)_2$), 103.1 ($CH(O)_2$), 128.2 (2xCH_{arom}), 129.5 (CH_{arom}), 129.8 (C_{arom}), 132.5 (2xCH_{arom}). **MS (ESI): m/z** Exact mass calculated for $C_{29}H_{50}NO_{12}$ [M+H$^+$]: 604.3328. Found: 604.3353.

***N,N*-dimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (230e):** (0.28 g, 91%), **IR (cm^{-1}) υ_{max}:** 725, 906, 1030 and 1073 (CHOCH), 3359 (OH). **^{1}H-NMR (400MHz, MeOD):** δ_H 0.89 (3H, t, *J*=7.4 Hz, CH_2CH_3), 1.16 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.19-1.43 (11H, m, 5x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.46-1.55 (1H, m, $CH_aH_bCHCH_3$), 1.67-1.82 (4H, m, 2xCH_2CH_2N), 2.95-3.23 (9H, m, 2xCH_2CH_2N, 5xCHOC), 3.27 (1H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, CHOC), 3.37 (1H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, CHOC), 3.46 (1H, dxd, *J*=8.5 Hz, *J*=8.5 Hz, CHOC), 3.52-3.58 (2H, m, 2x$CHCH_aH_bOH$), 3.70-3.78 (3H, m, 2x$CHCH_aH_bOH$, CHOC), 4.31 (2H, s, $C_{arom}CH_2N$), 4.34 (1H, d, *J*=7.7 Hz, $CH(O)_2$), 4.56 (1H, d, *J*=7.8 Hz, $CH(O)_2$), 7.31-7.38 (3H, m, 3xCH_{arom}), 7.48-7.51 (2H, m, 2xCH_{arom}). **^{13}C-NMR (100MHz, MeOD):** δ_C 12.7 (CH_2CH_3), 19.5 (CH_2CH_3), 20.6 ($CHCH_3$), 22.6 (CH_2CH_2N), 24.7 (CH_2CH_2N, $CH_2(CH_2)_2$), 26.3 ($CH_2(CH_2)_2$), 29.0 ($CH_2(CH_2)_2$), 29.3 ($CH_2(CH_2)_2$), 36.4 (CH_2CHCH_3), 61.4 (CH_2OH), 61.7 (CH_2OH), 64.2 (CH_2CH_2N), 64.6 (CH_2CH_2N), 69.2 ($C_{arom}CH_2N$), 70.1 (CHOC), 70.4 (CHOC), 74.6 (CHOC), 76.4 (CHOC), 76.4 (CHOC), 76.9 (CHOC), 77.0 (CHOC), 77.6 (CHOC), 80.2 (CHOC), 101.5 ($CH(O)_2$), 103.1 ($CH(O)_2$), 128.2 (2xCH_{arom}), 129.4 (CH_{arom}), 129.7 (C_{arom}), 132.3 (2xCH_{arom}). **MS (ESI): m/z** Exact mass calculated for $C_{32}H_{56}NO_{12}$ [M+H$^+$]: 646.3797. Found: 646.3813.



230e

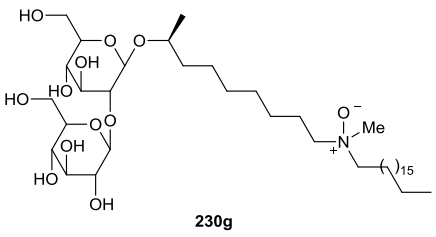
***N,N*-dibenzyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (230f):** (0.036 g, quant.), **^{1}H-NMR (400MHz, MeOD):** δ_H 1.14-1.21 (2H, m, $CH_2CH_2CH_2N$), 1.24 (3H, d, *J*=6.2 Hz, $CHCH_3$), 1.29-1.48 (7H, m, 3x$CH_2(CH_2)_2$, $CH_aH_bCHCH_3$), 1.55-1.62 (1H, m, $CH_aH_bCHCH_3$), 1.89-1.97 (2H, m, CH_2CH_2N), 2.85-2.90 (2H, m, CH_2CH_2N), 3.21-3.33 (5H, m, 5xCHOC), 3.38 (1H, dxd, *J*=8.9 Hz, *J*=8.9Hz, CHOC), 3.46 (1H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, 3.56 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, CHOC), 3.62-3.68 (2H, m, 2x$CHCH_aH_bOH$), 3.78-3.87 (3H, m, 2x$CHCH_aH_bOH$, CHOC), 4.38 (2H, d, *J*=12.7 Hz, 2x$C_{arom}CH_aH_bN$), 4.44 (1H, d, *J*=7.7 Hz, $CH(O)_2$), 4.48 (2H, d, *J*=12.7 Hz, 2x$C_{arom}CH_aH_bN$), 4.65 (1H, d, *J*=7.8 Hz, $CH(O)_2$), 7.41-7.45 (6H, m, 6xCH_{arom}), 7.62-7.64 (4H, m, 4xCH_{arom}). **^{13}C-NMR (100MHz, MeOD):** δ_C 20.6 ($CHCH_3$), 23.1 (CH_2CH_2N), 24.7 ($CH_2(CH_2)_2$), 26.2 ($CH_2(CH_2)_2$), 28.9 ($CH_2(CH_2)_2$), 29.2 ($CH_2(CH_2)_2$), 36.4 (CH_2CHCH_3), 61.4 (CH_2OH), 61.7 (CH_2OH), 63.7 (CH_2CH_2N), 69.7 (2x$C_{arom}CH_2N$), 70.1 (CHOC), 70.5 (CHOC), 74.6 (CHOC), 76.4 (CHOC), 76.4 (CHOC), 76.9 (CHOC), 76.9 (CHOC), 77.5 (CHOC),



230f

80.3 (CHOC), 101.5 ($\underline{C}H(O)_2$), 103.2 ($\underline{C}H(O)_2$), 128.2 (4x$\underline{C}H_{arom}$), 129.3 (2x$\underline{C}H_{arom}$), 130.1 (2x$\underline{C}_{arom}$), 132.4 (4x$\underline{C}H_{arom}$). **MS (ESI): m/z** Exact mass calculated for $C_{35}H_{54}NO_{12}$ [M+H$^+$]: 680.3641. Found: 680.3654.

***N*,*N*-dimethyl-(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonan-1-amine oxide (230g):** (0.34 g, 81%), **IR (cm^{-1}) υ_{max}:** 1030 and 1073 (CHOCH), 3346 (OH). **^{1}H-NMR (400MHz, MeOD):**



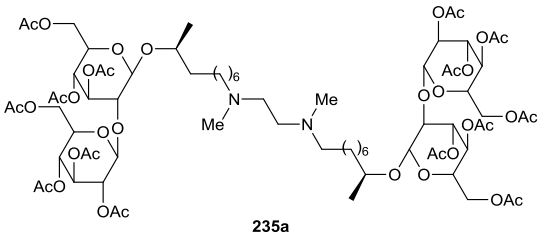
230g

δ_H 0.80 (3H, t, *J*=6.8 Hz, $CH_2C\underline{H}_3$), 1.15 (3H, d, *J*=6.2 Hz, $CHC\underline{H}_3$), 1.19-1.43 (39H, m, 19x$C\underline{H}_2(CH_2)_2$, $C\underline{H}_aH_bCHCH_3$), 1.46-1.54 (1H, m, $CH_a\underline{H}_bCHCH_3$), 1.66-1.76 (4H, m, 2x$C\underline{H}_2CH_2N$), 3.01 (3H, s, $C\underline{H}_3N$), 3.10-3.23 (7H, m, $CH_2C\underline{H}_2N$, 5x$C\underline{H}OC$), 3.28 (1H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, $C\underline{H}OC$), 3.34-3.39 (1H, m, $C\underline{H}OC$), 3.46 (1H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, $C\underline{H}OC$), 3.52-3.58 (2H, m, 2x$CHC\underline{H}_aH_bOH$), 3.69-3.77 (3H, m, 2x$CHCH_a\underline{H}_bOH$, $C\underline{H}OC$), 4.34 (1H, d, *J*=7.7 Hz, $C\underline{H}(O)_2$), 4.57 (1H, d, *J*=7.8 Hz, $C\underline{H}(O)_2$). **^{13}C-NMR (100MHz, MeOD):** δ_C 13.1 ($CH_2\underline{C}H_3$), 20.6 ($CH\underline{C}H_3$), 22.3 ($\underline{C}H_2CH_3$), 22.7 (2x$\underline{C}H_2CH_2N$), 24.7 ($\underline{C}H_2(CH_2)_2$), 26.3 (2x$\underline{C}H_2(CH_2)_2$), 29.0-29.4 (14x$\underline{C}H_2(CH_2)_2$), 31.7 ($\underline{C}H_2(CH_2)_2$), 36.5 ($\underline{C}H_2CHCH_3$), 53.6 ($\underline{C}H_3N$),61.4 ($\underline{C}H_2OH$), 61.7 ($\underline{C}H_2OH$), 68.3 (2x$CH_2\underline{C}H_2N$), 70.1 ($\underline{C}HOC$), 70.4 ($\underline{C}HOC$), 74.6 ($\underline{C}HOC$), 76.4 (2x$\underline{C}HOC$), 76.9 ($\underline{C}HOC$), 77.0 ($\underline{C}HOC$), 77.6 ($\underline{C}HOC$), 80.1 ($\underline{C}HOC$), 101.6 ($\underline{C}H(O)_2$), 103.1 ($\underline{C}H(O)_2$). **MS (ESI): m/z** Exact mass calculated for $C_{40}H_{80}NO_{12}$ [M+H$^+$]: 766.5675. Found: 766.5705.

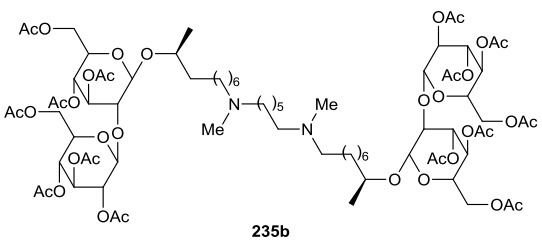
4.2.5. Synthesis of bolaamphiphilic sophorolipids

General procedure for the synthesis of peracetylated *N*,*N*'-dialkyl bolaamphiphilic sophorolipid amines (235): In a 50 mL flask, 1.57 g peracetylated sophoroplipid aldehyde **201** (2.02 mmol, 1 eq) was dissolved in 25 mL methanol and the secondary diamine (1.01 mmol, 1 eq), 0.25 g $NaBH_3CN$ (4.04 mmol, 2 eq) and 0.58 mL acetic acid (10.09 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The peracetylated sophorolipid amines were purified by automated column chromatography as a viscous colorless oil with a hexane/ethyl acetate/triethylamine mixture as eluent (mixture A = 16% triethylamine in ethyl acetate).

***N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diamine (235a):** Purification gradient: 2 CV 15% mixture A, 20 CV 15-60% mixture A, 2 CV 60% mixture A. (0.26 g, 31%), colorless viscous oil. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (6H, d, *J*=6.1 Hz, 2x$C\underline{H}_3CH$), 1.26-1.41 (18H, m, 2x$C\underline{H}_aH_bCHCH_3$, 8x$C\underline{H}_2(CH_2)_2$), 1.45-1.52 (4H, m, 2x$C\underline{H}_2CH_2N$), 1.56-1.63 (2H, m, 2x$CH_a\underline{H}_bCHCH_3$), 1.99 (6H, s, 2x$C\underline{H}_3C$=O), 2.00 (6H, s, 2x$C\underline{H}_3C$=O), 2.01 (6H, s, 2x$C\underline{H}_3C$=O), 2.03 (6H, s, 2x$C\underline{H}_3C$=O), 2.06 (6H, s, 2x$C\underline{H}_3C$=O), 2.08 (12H, s, 4x$C\underline{H}_3C$=O), 2.24 (6H, s, 2x$C\underline{H}_3N$), 2.34-2.37 (4H, m, 2x$C\underline{H}_2N$), 2.48 (4H, s, 2x$C\underline{H}_2N$), 3.63-3.75 (8H, m, 4x$C\underline{H}CH_2OAc$, 2x$CH_3C\underline{H}O$, 2x$C\underline{H}OC$), 4.06-4.10 (4H, m, 4x$CHC\underline{H}_aH_bOAc$), 4.22-4.31 (4H, m, 4x$CHCH_a\underline{H}_bOAc$), 4.48 (2H, d, *J*=7.6 Hz, 2x$C\underline{H}(O)_2$), 4.73 (2H, d, *J*=8.0 Hz, 2x$C\underline{H}(O)_2$), 4.88-4.93 (2H, m, 2x$C\underline{H}OC$), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz 2x$C\underline{H}OC$), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2x$C\underline{H}OC$), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2x$C\underline{H}OC$), 5.16 (2H, dxd, *J*=9.5 H, *J*=9.5 Hz, 2x$C\underline{H}OC$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (2x$C\underline{H}_3C$=O), 20.6 (6x$C\underline{H}_3C$=O), 20.7 (4x$C\underline{H}_3C$=O), 20.8 (2x$C\underline{H}_3C$=O), 21.2 (2x$C\underline{H}_3CH$), 25.1 (2x$C\underline{H}_2(CH_2)_2$), 27.3 (2x$C\underline{H}_2CH_2N$), 27.7 (2x$C\underline{H}_2(CH_2)_2$), 29.7 (2x$C\underline{H}_2(CH_2)_2$), 29.8 (2x$C\underline{H}_2(CH_2)_2$), 36.4 (2x$C\underline{H}_2CHCH_3$), 42.6 (2x$C\underline{H}_3N$), 55.6 (2x$C\underline{H}_2N$), 58.5 (2x$C\underline{H}_2N$), 62.0 (2x$C\underline{H}_2OAc$), 62.3 (2x$C\underline{H}_2OAc$), 68.3 (2x$C\underline{H}OC$), 68.9 (2x$C\underline{H}OC$), 71.3 (2x$C\underline{H}OC$), 71.7 (2x$C\underline{H}OC$), 71.8 (2x$C\underline{H}OC$), 73.1 (2x$C\underline{H}OC$), 74.6 (2x$C\underline{H}OC$), 77.7 (2x$C\underline{H}OC$), 77.9 (2x$C\underline{H}OC$), 100.4 (2x$C\underline{H}(O)_2$), 101.1 (2x$C\underline{H}(O)_2$), 169.3 (2x$CH_3\underline{C}$=O), 169.4 (2x$CH_3\underline{C}$=O), 169.8 (2x$CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.3 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{74}H_{117}N_2O_{36}$ [M+H$^+$]: 1609.7381. Found: 1609.7412.

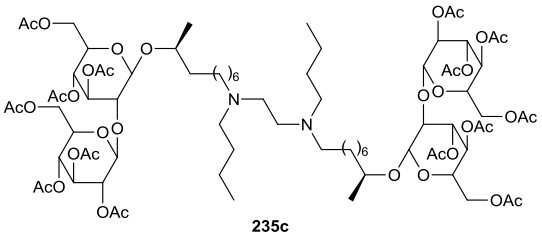


***N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diamine (235b):** Purification gradient: 2 CV 15% mixture A, 20 CV 15-60% mixture A, 2 CV 60% mixture A. (0.28 g, 31%), colorless viscous oil. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (6H, d, *J*=6.2 Hz, 2x$C\underline{H}_3CH$), 1.26-1.40 (20H, m, 2x$C\underline{H}_aH_bCHCH_3$, 9x$C\underline{H}_2(CH_2)_2$), 1.43-1.51 (8H, m, 4x$C\underline{H}_2CH_2N$), 1.56-1.63 (2H, m, 2x$CH_a\underline{H}_bCHCH_3$), 1.99 (6H, s, 2x$C\underline{H}_3C$=O), 2.00 (6H, s, 2x$C\underline{H}_3C$=O), 2.01 (6H, s, 2x$C\underline{H}_3C$=O), 2.03 (6H, s, 2x$C\underline{H}_3C$=O), 2.06 (6H, s, 2x$C\underline{H}_3C$=O), 2.08 (12H, s, 4x$C\underline{H}_3C$=O), 2.20 (6H, s, 2x$CH_3N$), 2.29-2.34 (8H, m, 4x$CH_2N$), 3.63-3.76 (8H, m, 4x$C\underline{H}CH_2OAc$, 2x$CH_3C\underline{H}O$, 2x$C\underline{H}OC$), 4.07-4.10 (4H, m, 4x$CHC\underline{H}_aH_bOAc$), 4.22-4.31 (4H, m, 4x$CHCH_a\underline{H}_bOAc$), 4.48 (2H, d, *J*=7.6 Hz, 2x$C\underline{H}(O)_2$), 4.73 (2H, d, *J*=8.0 Hz, 2x$C\underline{H}(O)_2$), 4.91 (2H, dxd, *J*=9.1 Hz, *J*=9.1 Hz 2x$C\underline{H}OC$), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz 2x$C\underline{H}OC$), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz 2x$C\underline{H}OC$), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz 2x$C\underline{H}OC$), 5.16



(2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz 2xCHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (2xCH$_3$C=O), 20.6 (6xCH$_3$C=O), 20.7 (4xCH$_3$C=O), 20.8 (2xCH$_3$C=O), 21.2 (2xCH$_3$CH), 25.1 (2xCH$_2$(CH$_2$)$_2$), 27.3 (2xCH$_2$CH$_2$N), 27.3 (2xCH$_2$CH$_2$N), 27.7 (2xCH$_2$(CH$_2$)$_2$), 27.7 (2xCH$_2$(CH$_2$)$_2$), 29.7 (2xCH$_2$(CH$_2$)$_2$), 29.8 (2xCH$_2$(CH$_2$)$_2$), 36.4 (2xCH$_2$CHCH$_3$), 42.2 (2xCH$_3$N), 57.9 (2xCH$_2$N), 58.0 (2xCH$_2$N), 68.2 (2xCHOC), 68.9 (2xCHOC), 71.3 (2xCHOC), 71.7 (2xCHOC), 71.8 (2xCHOC), 73.0 (2xCHOC), 74.6 (2xCHOC), 77.7 (2xCHOC), 77.9 (2xCHOC), 100.4 (2xCH(O)$_2$), 101.1 (2xCH(O)$_2$), 169.3 (2xCH$_3$C=O), 169.4 (2xCH$_3$C=O), 169.7 (2xCH$_3$C=O), 170.0 (2xCH$_3$C=O), 170.3 (2xCH$_3$C=O), 170.6 (2xCH$_3$C=O), 170.6 (2xCH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{78}H_{125}N_2O_{36}$ [M+H$^+$]: 1665.8007. Found: 1668.8089.

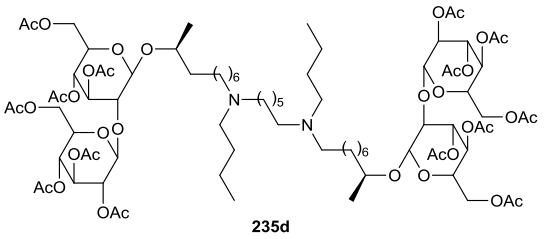
***N*,*N*'-dibutyl,*N*,*N*'-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diamine (235c):** Purification gradient: 2 CV 7% mixture A, 30 CV 7-60% mixture A, 2 CV 60% mixture A, 2 CV 100% mixture A. (0.43 g, 41%), colorless viscous oil.



235c

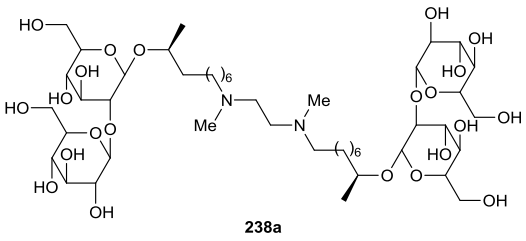
^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 0.91 (6H, t, *J*=7.3 Hz, 2xCH$_3$CH$_2$), 1.22 (6H, d, *J*=6.2 Hz, 2xCH$_3$CH), 1.24-1.48 (30H, m, 2xCH$_a$H$_b$CHCH$_3$, 2xCH$_2$CH$_3$, 8xCH$_2$(CH$_2$)$_2$, 4xCH$_2$CH$_2$N), 1.57-1.63 (2H, m, 2xCH$_a$H$_b$CHCH$_3$), 1.99 (6H, s, 2xCH$_3$C=O), 2.00 (6H, s, 2xCH$_3$C=O), 2.01 (6H, s, 2xCH$_3$C=O), 2.03 (6H, s, 2xCH$_3$C=O), 2.06 (6H, s, 2xCH$_3$C=O), 2.08 (6H, s, 2xCH$_3$C=O), 2.08 (6H, s, 2xCH$_3$C=O), 2.40-2.44 (8H, m, 4xCH$_2$N), 2.52 (4H, s, 2xCH$_2$N), 3.63-3.75 (8H, m, 4xCHCH$_2$OAc, 2xCH$_3$CHO, 2xCHOC), 4.07-4.10 (4H, m, 4xCHCH$_a$H$_b$OAc), 4.22-4.31 (4H, m, 4xCHCH$_a$H$_b$OAc), 4.48 (2H, d, *J*=7.6 Hz, 2xCH(O)$_2$), 4.73 (2H, d, *J*=8.0 Hz, 2xCH(O)$_2$), 4.91 (2H, dxd, *J*=9.2 Hz, *J*=9.2 Hz, 2xCHOC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xCHOC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xCHOC), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.34 Hz, 2xCHOC), 5.16 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xCHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 14.1 (2xCH$_3$CH$_2$), 20.5 (2xCH$_3$C=O), 20.6 (2xCH$_3$C=O), 20.6 (2xCH$_3$C=O), 20.6 (2xCH$_3$C=O), 20.7 (4xCH$_3$C=O), 20.8 (2xCH$_3$CH$_2$), 20.8 (2xCH$_3$C=O), 21.2 (2xCH$_3$CH), 25.1 (2xCH$_2$(CH$_2$)$_2$), 27.2 (2xCH$_2$CH$_2$N), 27.7 (2x CH$_2$(CH$_2$)$_2$), 29.3 (2xCH$_2$CH$_2$N), 29.8 (2xCH$_2$(CH$_2$)$_2$), 29.8 (2xCH$_2$(CH$_2$)$_2$), 36.4 (2xCH$_2$CHCH$_3$), 52.3 (2xCH$_2$N), 54.3 (2xCH$_2$N), 54.9 (2xCH$_2$N), 62.0 (2xCH$_2$OAc), 62.3 (2xCH$_2$OAc), 68.2 (2xCHOC), 68.9 (2xCHOC), 71.3 (2xCHOC), 71.7 (2xCHOC), 71.8 (2xCHOC), 73.1 (2xCHOC), 74.6 (2xCHOC), 77.7 (2xCHOC), 77.9 (2xCHOC), 100.4 (2xCH(O)$_2$), 101.1 (2xCH(O)$_2$), 169.3 (2xCH$_3$C=O), 169.4 (2xCH$_3$C=O), 169.7 (2xCH$_3$C=O), 170.0 (2xCH$_3$C=O), 170.3 (2xCH$_3$C=O), 170.6 (2xCH$_3$C=O), 170.6 (2xCH$_3$C=O). **MS (ESI): m/z** Exact mass calculated for $C_{74}H_{117}N_2O_{36}$ [M+H$^+$]: 1693.8320. Found: 1693.8377.

***N*,*N*'-dibutyl,*N*,*N*'-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diamine (235d):** Purification gradient: 2 CV 7% mixture A, 30 CV 7-60% mixture A, 2 CV 60% mixture A, 2 CV 100% mixture A. (0.29 g, 27%), colorless viscous oil. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.91 (6H, t, *J*=7.3 Hz, 2xC$\underline{H}_3$CH$_2$), 1.22 (6H, d, *J*=6.2 Hz, 2xC$\underline{H}_3$CH), 1.26-1.47 (38H, m, 2xC$\underline{H}_a$H$_b$CHCH$_3$, 2xC$\underline{H}_2$CH$_3$, 10xC$\underline{H}_2$(CH$_2$)$_2$, 6xC$\underline{H}_2$CH$_2$N), 1.57-1.63 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.01 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (12H, s, 4xC$\underline{H}_3$C=O), 2.37-2.41 (12H, m, 6xC$\underline{H}_2$N), 3.63-3.75 (8H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC), 4.06-4.10 (4H, m, 4xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.31 (4H, m, 4xCHCH$_a\underline{H}_b$OAc), 4.48 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.73 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}$(O)$_2$), 4.91 (2H, dxd, *J*=9.3 Hz, *J*=8.3 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.13 (2H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, 2xC$\underline{H}$OC), 5.16 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 14.1 (2x$\underline{C}$H$_3$CH$_2$), 20.5 (2x$\underline{C}$H$_3$C=O), 20.5 (2x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.7 (4x$\underline{C}$H$_3$C=O), 20.8 (2x$\underline{C}$H$_3$C=O), 20.8 (2xCH$_3\underline{C}$H$_2$), 21.2 (2x$\underline{C}$H$_3$CH), 25.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 27.0 (2x$\underline{C}$H$_2$CH$_2$N), 27.1 (2x$\underline{C}$H$_2$CH$_2$N), 27.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 27.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.2 (2x$\underline{C}$H$_2$CH$_2$N), 29.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.4 (2x$\underline{C}$H$_2$CHCH$_3$), 53.8 (2x$\underline{C}$H$_2$N), 54.2 (2x$\underline{C}$H$_2$N), 54.3 (2x$\underline{C}$H$_2$N), 62.0 (2x$\underline{C}$H$_2$OAc), 62.3 (2x$\underline{C}$H$_2$OAc), 68.2 (2x$\underline{C}$HOC), 68.9 (2x$\underline{C}$HOC), 71.3 (2x$\underline{C}$HOC), 71.7 (2x$\underline{C}$HOC), 71.8 (2x$\underline{C}$HOC), 73.0 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 77.9 (2x$\underline{C}$HOC), 100.4 (2x$\underline{C}$H(O)$_2$), 101.1 (2x$\underline{C}$H(O)$_2$), 169.3 (2xCH$_3\underline{C}$=O), 169.4 (2xCH$_3\underline{C}$=O), 169.7 (2xCH$_3\underline{C}$=O), 170.0 (2xCH$_3\underline{C}$=O), 170.3 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O). **MS (ESI): m/z** Exact mass calculated for $C_{74}H_{117}N_2O_{36}$ [M+H$^+$]: 1749.8946. Found: 1749.8995.

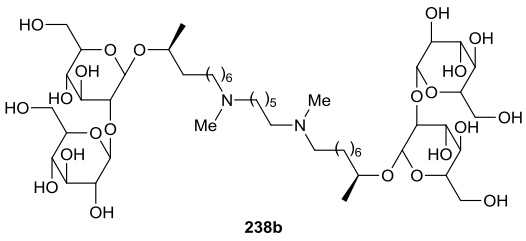


General procedure for the synthesis of deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipid amines (238): In a 50 mL flask, *N*,*N*'-dialkyl bolaamphiphilic sophorolipid **235** (0.45 mmol, 1 eq) was dissolved in a methanol/water mixture (1:1) and 13 mL Et_3N (0.90 mmol, 2 eq) was added. The mixture was stirred for 2 h at reflux temperature and concentrated under reduced pressure to yield pure deprotected *N*,*N*'-dialkyl bolaamphiphilic sophorolipid **238**.

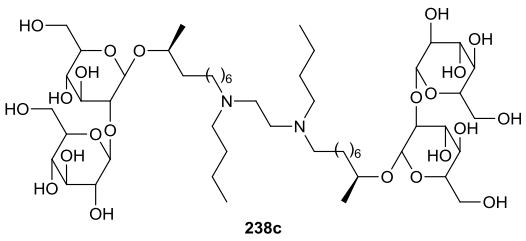
***N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diamine (238a):** (0.12 g, 94%), colorless sticky solid. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.27 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.31-1.56 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.58-1.67 (6H, m, 2xC$\underline{H}_2$CH$_2$N, 2xCH$_a\underline{H}_b$CHCH$_3$), 2.52 (6H, s, 2xC$\underline{H}_3$N), 2.68-2.74 (4H, m, 2xCH$_2$C$\underline{H}_2$N), 2.89 (4H, s, 2x C$\underline{H}_2$N), 3.22-3.52 (10H, m, 10xC$\underline{H}$OC), 3.40 (2H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, 2xC$\underline{H}$OC), 3.49 (2H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, 2xC$\underline{H}$OC), 3.58 (2H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, 2xC$\underline{H}$OC), 3.65-3.70 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.81-3.89 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.46 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.69 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 20.6 (2xCH$\underline{C}$H$_3$), 24.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 25.4 (2x$\underline{C}$H$_2$CH$_2$N), 26.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.2 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 40.4 (2x$\underline{C}$H$_3$N), 52.5 (2x$\underline{C}$H$_2$N), 57.2 (2xCH$_2\underline{C}$H$_2$N), 61.4 (2x$\underline{C}$H$_2$OH), 61.7 (2x$\underline{C}$H$_2$OH), 70.1 (2x$\underline{C}$HOC), 70.5 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.4 (4x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.0 (2x$\underline{C}$HOC), 77.6 (2x$\underline{C}$HOC), 80.2 (2x$\underline{C}$HOC), 101.5 (2x$\underline{C}$H(O)$_2$), 103.1 (2x$\underline{C}$H(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{46}H_{89}N_2O_{22}$ [M+H$^+$]: 1021.5902. Found: 1021.5899.



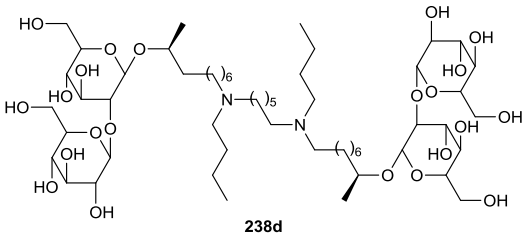
***N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diamine (238b):** (0.14 g, 95%), colorless sticky solid. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.26 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.29-1.54 (22H, m, 10xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.57-1.73 (10H, m, 4xC$\underline{H}_2$CH$_2$N, 2xCH$_a\underline{H}_b$CHCH$_3$), 2.67 (6H, s, 2xC$\underline{H}_3$N), 2.88-2.93 (8H, m, 4xCH$_2$C$\underline{H}_2$N), 3.20-3.33 (10H, m, 10xC$\underline{H}$OC), 3.39 (2H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, 2xC$\underline{H}$OC), 3.48 (2H, dxd, *J*=9.0 Hz, *J*=7.7 Hz, 2xC$\underline{H}$OC), 3.56 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.64-3.68 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.80-3.88 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.45 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}$(O)$_2$), 4.68 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 20.6 (2xCH$\underline{C}$H$_3$), 24.3 (2x$\underline{C}$H$_2$CH$_2$N), 24.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 24.7 (2x$\underline{C}$H$_2$CH$_2$N), 26.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 39.5 (2x$\underline{C}$H$_3$N), 56.1 (2xCH$_2\underline{C}$H$_2$N), 56.3 (2xCH$_2\underline{C}$H$_2$N), 61.4 (2x$\underline{C}$H$_2$OH), 61.6 (2x$\underline{C}$H$_2$OH), 70.2 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.4 (4x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.0 (2x$\underline{C}$HOC), 77.6 (2x$\underline{C}$HOC), 80.1 (2x$\underline{C}$HOC), 101.6 (2x$\underline{C}$H(O)$_2$), 103.1 (2x$\underline{C}$H(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{50}H_{97}N_2O_{22}$ [M+H$^+$]: 1077.6528. Found: 1077.6520.



***N,N'*-dibutyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diamine (238c):** (0.12 g, 91%), white sticky solid. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.00 (6H, t, *J*=7.3 Hz, 2xCH$_2$C<u>H</u>$_3$), 1.27 (6H, d, *J*=6.2 Hz, 2xCHC<u>H</u>$_3$), 1.31-1.51 (22H, m, 2xC<u>H</u>$_2$CH$_3$, 8xC<u>H</u>$_2$(CH$_2$)$_2$, 2xC<u>H</u>$_a$H$_b$CHCH$_3$), 1.53-1.66 (10H, m, 4xC<u>H</u>$_2$CH$_2$N, 2xCH$_a$<u>H</u>$_b$CHCH$_3$), 2.75-2.79 (8H, m, 4xCH$_2$C<u>H</u>$_2$N), 2.92 (4H, s, 2xC<u>H</u>$_2$N), 3.22-3.37 (10H, m, 10xC<u>H</u>OC), 3.40 (2H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, 2xC<u>H</u>OC), 3.48 (2H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, 2xC<u>H</u>OC), 3.58 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC<u>H</u>OC), 3.65-3.70 (4H, m, 4xCHC<u>H</u>$_a$H$_b$OH), 3.82-3.90 (6H, m, 4xCHCH$_a$<u>H</u>$_b$OH, 2xC<u>H</u>OC), 4.46 (2H, d, *J*=7.7 Hz, 2xC<u>H</u>(O)$_2$), 4.68 (2H, d, *J*=7.8 Hz, 2xC<u>H</u>(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.9 (2xCH$_2$<u>C</u>H$_3$), 20.1 (2x<u>C</u>H$_2$CH$_3$), 20.6 (2xCH<u>C</u>H$_3$), 24.8 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 25.2 (2x<u>C</u>H$_2$CH$_2$N), 27.0 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 27.3 (2x<u>C</u>H$_2$CH$_2$N), 29.3 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.5 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 36.5 (2x<u>C</u>H$_2$CHCH$_3$), 49.6 (2x<u>C</u>H$_2$N), 53.3 (2xCH$_2$<u>C</u>H$_2$N), 53.6 (2xCH$_2$<u>C</u>H$_2$N), 61.4 (2x<u>C</u>H$_2$OH), 61.7 (2x<u>C</u>H$_2$OH), 70.1 (2x<u>C</u>HOC), 70.4 (2x<u>C</u>HOC), 74.6 (2x<u>C</u>HOC), 76.4 (4x<u>C</u>HOC), 76.9 (4x<u>C</u>HOC), 77.6 (2x<u>C</u>HOC), 80.3 (2x<u>C</u>HOC), 101.5 (2x<u>C</u>H(O)$_2$), 103.2 (2x<u>C</u>H(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{52}H_{101}N_2O_{22}$ [M+H$^+$]: 1105.6841. Found: 1105.6834.

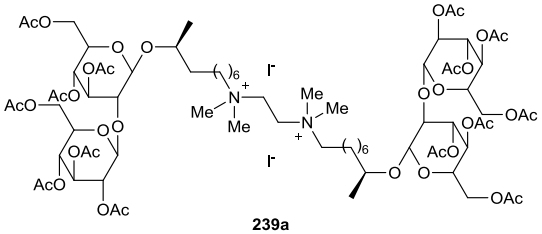

238c

***N,N'*-dibutyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diamine (238d):** (0.12 g, 89%), white sticky solid. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.01 (6H, t, *J*=7.3 Hz, 2xCH$_2$C<u>H</u>$_3$), 1.26 (6H, d, *J*=6.2 Hz, 2xCHC<u>H</u>$_3$), 1.20-1.55 (26H, m, 2xC<u>H</u>$_2$CH$_3$, 10xC<u>H</u>$_2$(CH$_2$)$_2$, 2xC<u>H</u>$_a$H$_b$CHCH$_3$), 1.58-1.72 (14H, m, 6xC<u>H</u>$_2$CH$_2$N, 2xCH$_a$<u>H</u>$_b$CHCH$_3$), 2.96-3.00 (12H, m, 6xCH$_2$C<u>H</u>$_2$N), 3.21-3.34 (10H, m, 10xC<u>H</u>OC), 3.40 (2H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, 2xC<u>H</u>OC), 3.48 (2H, dxd, *J*=9.0 Hz, *J*=7.8 Hz, 2xC<u>H</u>OC), 3.56 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC<u>H</u>OC), 3.64-3.69 (4H, m, 4xCHC<u>H</u>$_a$H$_b$OH), 3.81-3.88 (6H, m, 4xCHCH$_a$<u>H</u>$_b$OH, 2xC<u>H</u>OC), 4.45 (2H, d, *J*=7.6 Hz, 2xC<u>H</u>(O)$_2$), 4.68 (2H, d, *J*=7.8 Hz, 2xC<u>H</u>(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.6 (2xCH$_2$<u>C</u>H$_3$), 19.7 (2x<u>C</u>H$_2$CH$_3$), 20.6 (2xCH<u>C</u>H$_3$), 23.7 (2x<u>C</u>H$_2$CH$_2$N), 23.8 (2x<u>C</u>H$_2$CH$_2$N) 24.7 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 25.7 (2x<u>C</u>H$_2$CH$_2$N), 26.0 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 26.5 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.0 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.4 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 36.5 (2x<u>C</u>H$_2$CHCH$_3$), 52.4 (2x<u>C</u>H$_2$N), 52.5 (2x<u>C</u>H$_2$N), 52.8 (2x<u>C</u>H$_2$N), 61.4 (2x<u>C</u>H$_2$OH), 61.6 (2x<u>C</u>H$_2$OH), 70.1 (2x<u>C</u>HOC), 70.5 (2x<u>C</u>HOC), 74.7 (2x<u>C</u>HOC), 76.4 (4x<u>C</u>HOC), 76.9 (2x<u>C</u>HOC), 77.0 (2x<u>C</u>HOC), 77.6 (2x<u>C</u>HOC), 80.2 (2x<u>C</u>HOC), 101.6 (2x<u>C</u>H(O)$_2$), 103.1 (2x<u>C</u>H(O)$_2$). **MS (ESI): m/z** Exact mass calculated for $C_{56}H_{109}N_2O_{22}$ [M+H$^+$]: 1161.7467. Found: 1161.7458.


238d

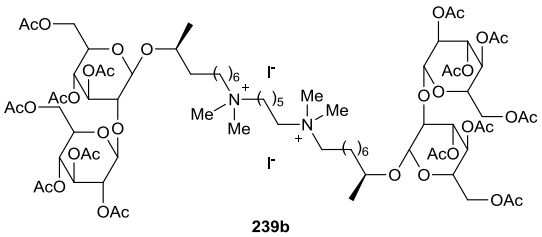
General procedure for the synthesis of peracetylated dicationic bolaamphiphilic sophorolipids (239): In a 10 mL flame dried pressure resistant vial, peracetylated *N*,*N*'dialkyl bolaamphiphilic sophorolipid **235** was dissolved in dry acetonitrile. The solution was cooled down to 0 °C and the alkyl iodide (10 eq) was added. The vial was closed and heated to 80 °C for 48 hours. The reaction mixture was concentrated under reduced pressure to yield the peracetylated quaternary ammonium bolaamphiphilic sophorolipid **239**.

***N*,*N*,*N*',*N*'-tetramethyl,*N*,*N*'-bis((*S*)-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl-ethylene diaminium diodide (239a):** (0.45 g, quant.), light yellow



powder. **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 1.22 (6H, d, *J*=6.1 Hz, 2xCHC$\underline{H}_3$), 1.26-1.51 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.52-1.57 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.85-1.95 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 2.00 (12H, s, 4xC$\underline{H}_3$C=O), 2.04 (6H, s, 2xC$\underline{H}_3$C=O), 2.05 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.09 (6H, s, 2xC$\underline{H}_3$C=O), 2.10 (6H, s, 2xC$\underline{H}_3$C=O), 3.54-3.55 (12H, m, 4xC$\underline{H}_3$N), 3.66-3.81 (10H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC, 2xC$\underline{H}_2$N), 4.06-4.11 (4H, m, 4xCHC$\underline{H}_a$H$_b$OAc), 4.21-4.27 (4H, m, 4xCHCH$_a\underline{H}_b$OAc), 4.51 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.71-4.73 (4H, m, 2xCH$_2$N), 4.72 (2H, d, *J*=7.9 Hz, 2xC$\underline{H}$(O)$_2$), 4.86 (2H, dxd, *J*=9.5 Hz, *J*=8.1 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 4.99 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.12 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2xC$\underline{H}$OC), 5.18 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 20.5 (2xC$\underline{H}_3$C=O), 20.6 (2xC$\underline{H}_3$C=O), 20.7 (2xC$\underline{H}_3$C=O), 20.8 (2xC$\underline{H}_3$C=O), 20.8 (4xC$\underline{H}_3$C=O), 21.0 (2xC$\underline{H}_3$C=O), 21.5 (2xCHC$\underline{H}_3$), 22.8 (2xC$\underline{H}_2$CH$_2$N), 24.6 (2xC$\underline{H}_2(CH_2)_2$), 26.0 (2xC$\underline{H}_2(CH_2)_2$), 29.3 (2xC$\underline{H}_2(CH_2)_2$), 29.3 (2xC$\underline{H}_2(CH_2)_2$), 36.5 (2xC$\underline{H}_2$CHCH$_3$), 51.4 (4xC$\underline{H}_3$N), 56.8 (C$\underline{H}_2$N), 62.2 (2xC$\underline{H}_2$OAc), 62.5 (2xC$\underline{H}_2$OAc), 66.2 (C$\underline{H}_2$N), 68.6 (2xC$\underline{H}$OC), 68.8 (2xC$\underline{H}$OC), 71.2 (2xC$\underline{H}$OC), 71.5 (2xC$\underline{H}$OC), 71.7 (2xC$\underline{H}$OC), 72.9 (2xC$\underline{H}$OC), 74.8 (2xC$\underline{H}$OC), 77.9 (2xC$\underline{H}$OC), 77.9 (2xC$\underline{H}$OC), 100.5 (2xC$\underline{H}$(O)$_2$), 101.2 (2xC$\underline{H}$(O)$_2$), 169.7 (2xCH$_3\underline{C}$=O), 169.8 (2xCH$_3\underline{C}$=O), 169.9 (2xCH$_3\underline{C}$=O), 170.0 (4xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O), 170.8 (2xCH$_3\underline{C}$=O).

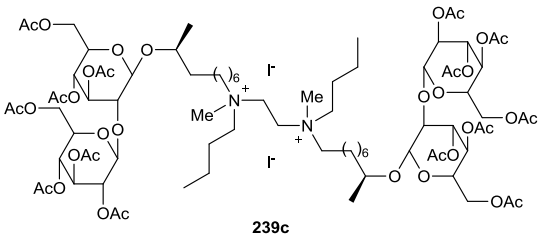
***N*,*N*,*N*',*N*'-tetramethyl,*N*,*N*'-bis((*S*)-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diaminium diodide (239b):** (0.40 g, quant.), yellow



powder. **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 1.22 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.25-1.47 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.51-1.58 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.59-1.66 (4H, m, 2xC$\underline{H}_2(CH_2)_2$), 1.77-1.85 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 1.98-2.09 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 1.99 (6H,

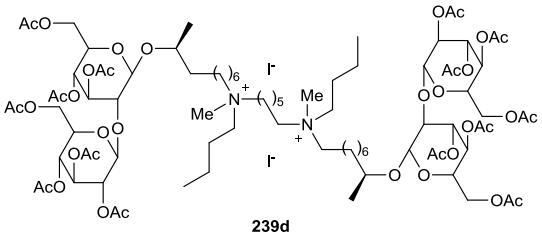
s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (12H, s, 4xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.09 (6H, s, 2xC$\underline{H}_3$C=O), 3.36-3.37 (12H, m, 4xC$\underline{H}_3$N), 3.45-3.49 (4H, m, 2xC$\underline{H}_2$N), 3.45-3.74 (10H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC, 2xC$\underline{H}_2$N), 4.06-4.11 (4H, m, 4xCHC$\underline{H}_a\underline{H}_b$OAc), 4.22-4.27 (4H, m, 4xCHCH$_a\underline{H}_b$OAc), 4.47 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.70 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}$(O)$_2$), 4.86 (2H, dxd, *J*=9.5 Hz, *J*=8.1 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.00 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.11 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.17 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 20.5 (2xC$\underline{C}$H$_3$C=O), 20.6 (2xC$\underline{C}$H$_3$C=O), 20.7 (2xC$\underline{C}$H$_3$C=O), 20.7 (2xC$\underline{C}$H$_3$C=O), 20.7 (2xC$\underline{C}$H$_3$C=O), 20.8 (4xC$\underline{C}$H$_3$C=O), 21.5 (2xCH$\underline{C}$H$_3$), 21.9 (2xC$\underline{C}$H$_2$CH$_2$N), 22.7 (2xC$\underline{C}$H$_2$CH$_2$N), 24.2 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 24.7 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 26.3 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 29.4 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2xC$\underline{C}$H$_2$CHCH$_3$), 51.2 (4xC$\underline{C}$H$_3$N), 62.2 (2xC$\underline{C}$H$_2$OAc), 62.3 (2xC$\underline{C}$H$_2$OAc), 64.4 (2xC$\underline{C}$H$_2$N), 65.2 (2xC$\underline{C}$H$_2$N), 68.5 (2xC$\underline{C}$HOC), 68.8 (2xC$\underline{C}$HOC), 71.2 (2xC$\underline{C}$HOC), 71.4 (2xC$\underline{C}$HOC), 71.7 (2xC$\underline{C}$HOC), 72.9 (2xC$\underline{C}$HOC), 74.8 (2xC$\underline{C}$HOC), 77.8 (2xC$\underline{C}$HOC), 78.0 (2xC$\underline{C}$HOC), 100.5 (2xC$\underline{C}$H(O)$_2$), 101.3 (2xC$\underline{C}$H(O)$_2$), 169.6 (4xCH$_3\underline{C}$=O), 169.7 (2xCH$_3\underline{C}$=O), 170.0 (2xCH$_3\underline{C}$=O), 170.0 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O).

***N*,*N'*-dibutyl,*N*,*N'*-dimethyl,*N*,*N'*-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diaminium diodide (239c):** (0.38 g, 93%),



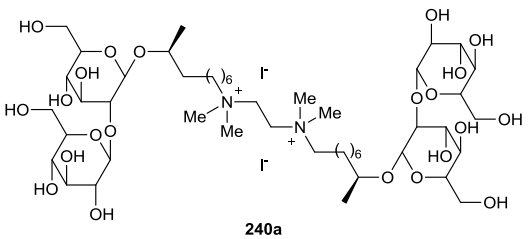
light yellow powder. **^{1}H-NMR (400 MHz, CDCl$_3$): δ_H** 0.98 (6H, t, *J*=7.3 Hz, 2xCH$_2$C$\underline{H}_3$), 1.15 (6H, d, *J*=6.1 Hz, 2xCHC$\underline{H}_3$), 1.19-1.56 (24H, m, 8xC$\underline{H}_2$(CH$_2$)$_2$, 2xC$\underline{H}_2$CHCH$_3$, 2xC$\underline{H}_2$CH$_3$), 1.71-1.82 (8H, m, 4xC$\underline{H}_2$CH$_2$N), 1.93 (12H, s, 4C$\underline{H}_3$C=O), 1.97 (12H, s, 4xC$\underline{H}_3$C=O), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.02 (6H, s, 2xC$\underline{H}_3$C=O), 2.02 (6H, s, 2xC$\underline{H}_3$C=O), 3.41-3.43 (6H, m, 2xC$\underline{H}_3$N), 3.60-3.71 (16H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC, 4xC$\underline{H}_2$N), 3.99-4.04 (4H, m, 4xCHC$\underline{H}_a$H$_b$OAc), 4.14-4.21 (4H, m, 4xCHCH$_a\underline{H}_b$OAc), 4.42-4.45 (2H, m, 2xC$\underline{H}$(O)$_2$), 4.52 (4H, s, 2xC$\underline{H}_2$N), 4.66 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$), 4.78-4.82 (2H, m, 2xC$\underline{H}$OC), 4.86 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.05 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2xC$\underline{H}$OC), 5.10 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 13.8 (2xCH$_2\underline{C}$H$_3$), 19.6 (2xC$\underline{C}$H$_2$CH$_3$), 20.6 (4xC$\underline{C}$H$_3$C=O), 20.7 (4xC$\underline{C}$H$_3$C=O), 20.8 (4xC$\underline{C}$H$_3$C=O), 20.9 (2xC$\underline{C}$H$_3$C=O), 21.5 (2xCH$\underline{C}$H$_3$), 22.7 (2xC$\underline{C}$H$_2$CH$_2$N), 24.7 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$, 2xC$\underline{C}$H$_2$CH$_2$N), 26.2 (2xC$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (4xC$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2xC$\underline{C}$H$_2$CHCH$_3$), 49.0 (2xC$\underline{C}$H$_3$N), 54.9 (2xC$\underline{C}$H$_2$N), 62.2 (4xC$\underline{C}$H$_2$OAc), 62.4 (4xC$\underline{C}$H$_2$N), 68.6 (2xC$\underline{C}$HOC), 68.8 (2xC$\underline{C}$HOC), 71.2 (2xC$\underline{C}$HOC), 71.5 (2xC$\underline{C}$HOC), 71.7 (2xC$\underline{C}$HOC), 72.9 (2xC$\underline{C}$HOC), 74.9 (2xC$\underline{C}$HOC), 77.9 (4xC$\underline{C}$HOC), 100.4 (2xC$\underline{C}$H(O)$_2$), 101.1 (2xC$\underline{C}$H(O)$_2$), 169.7 (6xCH$_3\underline{C}$=O), 170.0 (4xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O).

***N,N'*-dibutyl,*N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diaminium diodide (239d):** (0.41 g, 94%), yellow powder. **^{1}H-NMR (400 MHz, $CDCl_3$): δ_H** 1.02 (6H, t, *J*=7.3 Hz, 2x$CH_2C\underline{H}_3$), 1.22 (6H, d, *J*=6.2 Hz, 2x$CHC\underline{H}_3$), 1.26-1.52 (22H, m, 8x$C\underline{H}_2(CH_2)_2$, 2x$C\underline{H}_aH_bCHCH_3$, 2x$C\underline{H}_2CH_3$), 1.54-1.59 (2H, m, 2x$CH_a\underline{H}_bCHCH_3$), 1.62-1.67 (4H, m, 2x$C\underline{H}_2(CH_2)_2$), 1.68-1.78 (8H, m, 4x$C\underline{H}_2CH_2N$), 1.99-2.09 (4H, m, 2x$C\underline{H}_2CH_2N$), 1.99 (6H, s, 2x$C\underline{H}_3C$=O), 2.00 (6H, s, 2x$C\underline{H}_3C$=O), 2.03 (12H, s, 4x$C\underline{H}_3C$=O), 2.06 (6H, s, 2x$C\underline{H}_3C$=O), 2.08 (6H, s, 2x$C\underline{H}_3C$=O), 2.09 (6H, s, 2x$C\underline{H}_3C$=O), 3.32 (6H, m, 2x$C\underline{H}_3N$), 3.35-3.53 (8H, 4x$C\underline{H}_2N$), 3.61-3.74 (14H, m, 4x$C\underline{H}CH_2OAc$, 2x$CH_3C\underline{H}O$, 2x$C\underline{H}OC$, 2x$C\underline{H}_2N$), 4.05-4.11 (4H, m, 4x$CHC\underline{H}_aH_bOAc$), 4.22-4.27 (4H, m, 4x$CHCH_a\underline{H}_bOAc$), 4.46-4.48 (2H, m, 2x$C\underline{H}(O)_2$), 4.70 (2H, d, *J*=8.0 Hz, 2x$C\underline{H}(O)_2$), 4.87 (2H, dxd, *J*=9.5 Hz, *J*=8.2 Hz, 2x$C\underline{H}OC$), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2x$C\underline{H}OC$), 4.98-5.03 (2H, m, 2x$C\underline{H}OC$), 5.12 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2x$C\underline{H}OC$), 5.17 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2x$C\underline{H}OC$). **^{13}C-NMR (100 MHz, $CDCl_3$): δ_C** 13.8 (2x$CH_2\underline{C}H_3$), 19.7 (2x$\underline{C}H_2CH_3$), 20.5 (2x$\underline{C}H_3C$=O), 20.6 (2x$\underline{C}H_3C$=O), 20.7 (2x$\underline{C}H_3C$=O), 20.7 (2x$\underline{C}H_3C$=O), 20.7 (2x$\underline{C}H_3C$=O), 20.8 (4x$\underline{C}H_3C$=O), 21.5 (2x$CH\underline{C}H_3$), 21.7 (2x$\underline{C}H_2CH_2N$), 22.4 (2x$\underline{C}H_2CH_2N$), 24.3 (2x$\underline{C}H_2(CH_2)_2$), 24.4 (2x$\underline{C}H_2CH_2N$), 24.7 (2x$\underline{C}H_2(CH_2)_2$), 26.4 (2x$\underline{C}H_2(CH_2)_2$), 29.4 (2x$\underline{C}H_2(CH_2)_2$), 29.4 (2x$\underline{C}H_2(CH_2)_2$), 36.5 (2x$\underline{C}H_2CHCH_3$), 49.0 (2x$\underline{C}H_3N$), 61.4 (2x$\underline{C}H_2N$), 61.7 (2x$\underline{C}H_2N$), 62.2 (2x$\underline{C}H_2OAc$), 62.3 (2x$\underline{C}H_2OAc$), 62.7 (2x$\underline{C}H_2N$), 68.5 (2x$\underline{C}HOC$), 68.8 (2x$\underline{C}HOC$), 71.2 (2x$\underline{C}HOC$), 71.4 (2x$\underline{C}HOC$), 71.7 (2x$\underline{C}HOC$), 72.9 (2x$\underline{C}HOC$), 74.8 (2x$\underline{C}HOC$), 77.7 (2x$\underline{C}HOC$), 78.0 (2x$\underline{C}HOC$), 100.5 (2x$\underline{C}H(O)_2$), 101.3 (2x$\underline{C}H(O)_2$), 169.6 (4x$CH_3\underline{C}$=O), 169.7 (2x$CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.1 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O).



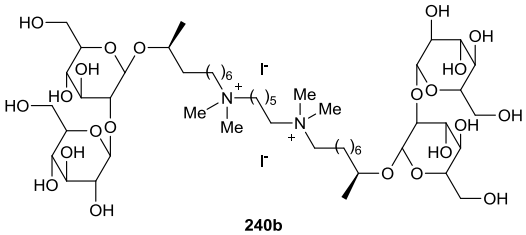
General procedure for the synthesis of deprotected dicationic bolaamphiphilic sophorolipids (240): In a 50 mL flask, peracetylated dicationic bolaamphiphilic sophorolipid **239** (0.45 mmol, 1 eq) was dissolved in a methanol/water mixture (1:1) and 13 mL Et_3N (0.90 mmol, 2 eq) was added. The mixture was stirred for 2 h at reflux temperature and concentrated under reduced pressure to yield pure dicationic bolaamphiphilic sophorolipid **240**.

***N,N,N',N'*-tetramethyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diaminium diodide (240a):** (0.16 g, 96%), light yellow powder. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.29 (6H, t, *J*=6.2 Hz, 2x$CHC\underline{H}_3$), 1.23-1.66 (20H, m, 8x$C\underline{H}_2(CH_2)_2$, 2x$C\underline{H}_2CHCH_3$), 1.88-1.96 (4H, m, $C\underline{H}_2CH_2N$), 3.23-3.40 (10H, m, 10x$C\underline{H}OC$), 3.35 (12H, s, 4x$C\underline{H}_3N$), 3.45 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2x$C\underline{H}OC$),

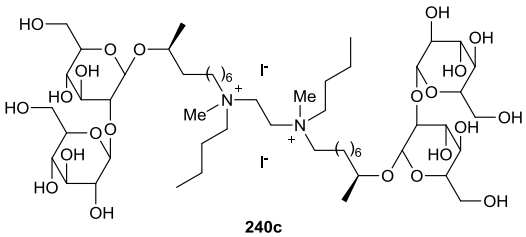


3.51-3.64 (8H, m, 4xCHOC, 2xCH$_2$N), 3.67-3.72 (4H, m, 4xCHCH$_a$H$_b$OH), 3.83-3.91 (6H, m, 4xCHCH$_a$H$_b$OH, 2xCHOC), 4.13 (4H, s, 2xCH$_2$N), 4.50 (2H, d, *J*=7.6 Hz, 2xCH(O)$_2$), 4.75 (2H, d, *J*=7.7 Hz, 2xCH(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.8 (2xCHCH$_3$), 22.5 (2xCH$_2$CH$_2$N), 24.6 (2xCH$_2$(CH$_2$)$_2$), 25.9 (2xCH$_2$(CH$_2$)$_2$), 29.1 (2xCH$_2$(CH$_2$)$_2$), 29.3 (2xCH$_2$(CH$_2$)$_2$), 36.6 (2xCH$_2$CHCH$_3$), 50.7 (4xCH$_3$N), 56.1 (4xCH$_2$N), 61.4 (2xCH$_2$OH), 61.6 (2xCH$_2$OH), 65.7 (2xCH$_2$N), 70.2 (2xCHOC), 70.5 (2xCHOC), 74.7 (2xCHOC), 76.4 (4xCHOC), 76.8 (2xCHOC), 77.1 (2xCHOC), 77.8 (2xCHOC), 79.5 (2xCHOC), 101.7 (2xCH(O)$_2$), 102.8 (2xCH(O)$_2$).

***N*,*N*,*N*',*N*'-tetramethyl,*N*,*N*'-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diaminium diodide (240b):** (0.15 g, 96%), yellow powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.29 (6H, t, *J*=6.2 Hz, 2xCHCH$_3$), 1.33-1.58 (22H, m, 10xCH$_2$(CH$_2$)$_2$, 2xCH$_a$H$_b$CHCH$_3$), 1.60-1.67 (2H, m, 2xCH$_a$H$_b$CHCH$_3$), 1.79-1.92 (8H, m, 4xCH$_2$CH$_2$N), 3.16 (12H, s, 4xCH$_3$N), 3.21-3.35 (10H, m, 10xCHOC), 3.37-3.45 (10H, m, 4xCH$_2$N, 2xCHOC), 3.49-3.54 (2H, m, 2xCHOC), 3.60 (2H, dxd, *J*=8.5 Hz, *J*=8.5 Hz, 2xCHOC), 3.67-3.71 (4H, m, 4xCHCH$_a$H$_b$OH), 3.84-3.91 (6H, m, 4xCHCH$_a$H$_b$OH, 2xCHOC), 4.49 (2H, d, *J*=7.7 Hz, 2xCH(O)$_2$), 4.72 (2H, d, *J*=7.8 Hz, 2xCH(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.7 (2xCHCH$_3$), 22.1 (2xCH$_2$CH$_2$N), 22.3 (2xCH$_2$CH$_2$N), 24.6 (2xCH$_2$(CH$_2$)$_2$), 25.4 (2xCH$_2$(CH$_2$)$_2$), 26.0 (2xCH$_2$(CH$_2$)$_2$), 29.0 (2xCH$_2$(CH$_2$)$_2$), 29.3 (2xCH$_2$(CH$_2$)$_2$), 36.5 (2xCH$_2$CHCH$_3$), 50.0 (4xCH$_3$N), 61.4 (2xCH$_2$OH), 61.6 (2xCH$_2$OH), 63.9 (2xCH$_2$N), 64.3 (2xCH$_2$N), 70.2 (2xCHOC), 70.4 (2xCHOC), 74.7 (2xCHOC), 76.4 (4xCHOC), 76.8 (2xCHOC), 77.0 (2xCHOC), 77.6 (2xCHOC), 80.0 (2xCHOC), 101.6 (2xCH(O)$_2$), 103.0 (2xCH(O)$_2$).

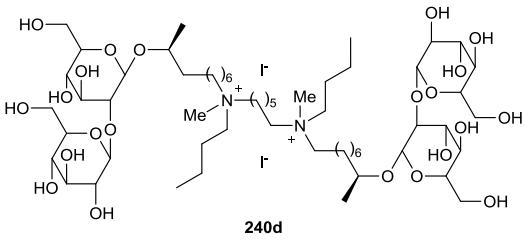

240b

***N*,*N'*-dibutyl,*N*,*N*'-dimethyl,*N*,*N*'-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl-ethylene diaminium diodide (240c):** (0.13 g, 79%), light yellow powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.08 (6H, t, *J*=7.3 Hz, 2xCH$_2$CH$_3$), 1.28 (6H, t, *J*=6.2 Hz, 2xCHCH$_3$), 1.32-1.56 (22H, m, 8xCH$_2$(CH$_2$)$_2$, 2xCH$_a$H$_b$CHCH$_3$, 2xCH$_2$CH$_3$), 1.58-1.67 (2H, m, 2xCH$_a$H$_b$CHCH$_3$), 1.79-1.89 (8H, m, 4xCH$_2$CH$_2$N), 3.22-3.37 (16H, m, 10xCHOC, 2xCH$_3$N), 3.39-3.62 (14H, m, 6xCHOC, 4xCH$_2$N), 3.66-3.70 (4H, m, 4xCHCH$_a$H$_b$OH), 3.82-3.89 (6H, m, 4xCHCH$_a$H$_b$OH, 2xCHOC), 4.03 (4H, s, 2xCH$_2$N), 4.48 (2H, d, *J*=7.6 Hz, 2xCH(O)$_2$), 4.71-4.73 (2H, m, 2xCH(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.8 (2xCH$_2$CH$_3$), 19.3 (2xCH$_2$CH$_2$CH$_3$), 20.8 (2xCHCH$_3$), 22.4 (2xCH$_2$CH$_2$N), 24.2 (2xCH$_2$CH$_2$N), 24.7 (2xCH$_2$(CH$_2$)$_2$), 26.0 (2xCH$_2$(CH$_2$)$_2$), 29.1 (2xCH$_2$(CH$_2$)$_2$), 29.4 (2xCH$_2$(CH$_2$)$_2$), 36.5 (2xCH$_2$CHCH$_3$), 48.4 (2xCH$_3$N), 54.0 (2xCH$_2$N), 61.4 (2xCH$_2$OH), 61.7 (2xCH$_2$OH), 62.3 (2xCH$_2$N), 62.6


240c

(2x$\underline{C}H_2N$), 70.2 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.3 (4x$\underline{C}$HOC), 76.8 (2x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 79.9 (2x$\underline{C}$HOC), 101.5 (2x$\underline{C}H(O)_2$), 102.9 (2x$\underline{C}H(O)_2$).

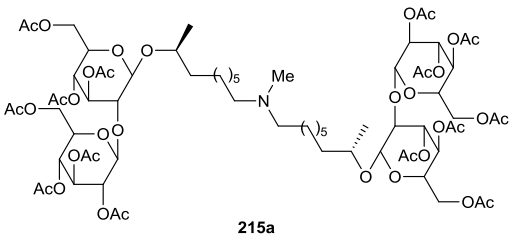
***N,N'*-dibutyl,*N,N'*-dimethyl,*N,N'*-bis(*(S)*-8-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl-hexamethylene diaminium diodide (240d):** (0.18 g, 94%), yellow powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.07 (6H, t, *J*=7.3 Hz, 2xCH$_2$C$\underline{H}_3$), 1.29 (6H, t, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.32-1.58 (26H, m, 10xC$\underline{H}_2$(CH$_2$)$_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$, 2xC$\underline{H}_2$CH$_3$), 1.59-1.69 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.72-1.89 (12H, m, 6xC$\underline{H}_2$CH$_2$N), 3.11 (6H, s, 2xC$\underline{H}_3$N), 3.26 (2H, dxd, *J*=9.2 Hz, *J*=7.9 Hz, 2xC$\underline{H}$OC), 3.29-3.47 (22H, m, 4xC$\underline{H}_2$N, 2xC$\underline{H}$OC, 6xC$\underline{H}_2$N), 3.51 (2H, dxd, *J*=9.1 Hz, *J*=7.7 Hz, 2xC$\underline{H}$OC), 3.60 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.67-3.71 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.84-3.91 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.49 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}(O)_2$), 4.71 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 12.7 (2xCH$_2\underline{C}$H$_3$), 19.4 (2x$\underline{C}$H$_2$CH$_3$), 20.7 (2xCH$\underline{C}$H$_3$), 21.9 (2x$\underline{C}$H$_2$CH$_2$N), 22.0 (2x$\underline{C}$H$_2$CH$_2$N), 23.9 (2x$\underline{C}$H$_2$CH$_2$N), 24.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 25.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 47.6 (2x$\underline{C}$H$_3$N), 61.4 (2x$\underline{C}$H$_2$OH, 4x$\underline{C}$H$_2$N), 61.6 (2x$\underline{C}$H$_2$OH, 2x$\underline{C}$H$_2$N), 70.2 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.4 (4x$\underline{C}$HOC), 76.7 (2x$\underline{C}$HOC), 77.0 (2x$\underline{C}$HOC), 77.6 (2x$\underline{C}$HOC), 80.1 (2x$\underline{C}$HOC), 101.5 (2x$\underline{C}H(O)_2$), 103.1 (2x$\underline{C}H(O)_2$).



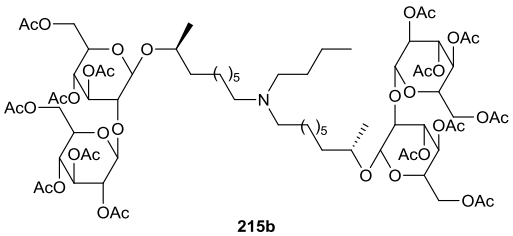
240d

General procedure for the synthesis of peracetylated *N*-alkyl bolaamphiphilic sophorolipids (215): In a 50 mL flask, 1.57 g peractylated sophoroplipid aldehyde **201** (2.02 mmol, 1 eq) was dissolved in 25 mL methanol and the primary amine (1.01 mmol, 0.5 eq), 0.25 g $NaBH_3CN$ (4.04 mmol, 2 eq) and 0.58 mL acetic acid (10.09 mmol, 5 eq) were added sequentially. The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and dissolved in ethyl acetate. The mixture was washed 3 times with a saturated $NaHCO_3$-solution and the organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The peracetylated *N*-methyl bolaamphiphilic sophorolipid **215a** was purified by automated column chromatography with a hexane/ethyl acetate/trimethylamine mixture as eluent (mixture A = 16% triethylamine in ethyl acetate). The other three derivatives **215b-d** were purified by preparative TLC with ethyl acetate as eluent.

***N*-methyl,*N*,*N*-bis(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (215a):** Purification gradient: 2 CV 10% mixture A, 30 CV 10-60% mixture A, 5 CV 60% mixture A, 10 CV 100% mixture A. (0.52 g, 55%), white sticky solid. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (6H, t, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.26-1.42 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a H_b CHCH_3$), 1.45-1.53 (4H, m, 2xC$\underline{H}_2CH_2N$), 1.56-1.63 (2H, m, 2xC$H_a\underline{H}_b CHCH_3$), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.01 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (12H, s, 4xC$\underline{H}_3$C=O), 2.23 (3H, s, C$\underline{H}_3$N), 2.33-2.37 (4H, m, 2xC$\underline{H}_2$N), 3.63-3.75 (8H, m, 4xC$\underline{H}CH_2OAc$, 2xC$H_3C\underline{H}O$, 2xC$\underline{H}OC$), 4.06-4.11 (4H, m, 4xCHC$H_a\underline{H}_b OAc$), 4.22-4.31 (4H, m, 4xCHC$H_a\underline{H}_b OAc$), 4.48 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}(O)_2$), 4.73 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}(O)_2$), 4.91 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xC$\underline{H}$OC), 5.16 (2H, dxd, *J*=9.5 H, *J*=9.5 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (2x$\underline{C}H_3$C=O), 20.6 (4x$\underline{C}H_3$C=O), 20.6 (2x$\underline{C}H_3$C=O), 20.7 (4x$\underline{C}H_3$C=O), 20.8 (2x$\underline{C}H_3$C=O), 21.2 (2xCH$\underline{C}H_3$), 25.0 (2x$\underline{C}H_2(CH_2)_2$), 27.1 (2x$\underline{C}H_2CH_2N$), 27.7 (2x$\underline{C}H_2(CH_2)_2$), 29.7 (2x$\underline{C}H_2(CH_2)_2$), 29.8 (2x$\underline{C}H_2(CH_2)_2$), 36.4 (2x$\underline{C}H_2CHCH_3$), 42.0 ($\underline{C}H_3N$), 57.8 (2x$\underline{C}H_2N$), 62.0 (2x$\underline{C}H_2OAc$), 62.2 (2x$\underline{C}H_2OAc$), 68.2 (2x$\underline{C}$HOC), 68.8 (2x$\underline{C}$HOC), 71.3 (2x$\underline{C}$HOC), 71.7 (2x$\underline{C}$HOC), 71.8 (2x$\underline{C}$HOC), 73.0 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 77.8 (2x$\underline{C}$HOC), 100.4 (2x$\underline{C}H(O)_2$), 101.1 (2x$\underline{C}H(O)_2$), 169.3 (2x$CH_3\underline{C}$=O), 169.4 (2x$CH_3\underline{C}$=O), 169.7 (2x$CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.3 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O).

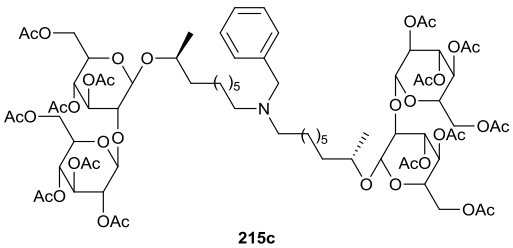


***N*-butyl,*N*,*N*-bis(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (215b)**: (0.15 g, 15%), white sticky solid. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.91 (3H, t, *J*=7.3 Hz, $CH_2C\underline{H}_3$), 1.22 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.24-1.48 (26H, m, 2xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a H_b CHCH_3$, C$\underline{H}_2CH_3$, 3xC$\underline{H}_2CH_2N$), 1.56-1.63 (2H, m, 2xC$H_a\underline{H}_b CHCH_3$), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.01 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (12H, s, 4xC$\underline{H}_3$C=O), 2.38-2.42 (6H, m, 3xC$\underline{H}_2$N), 3.64-3.75 (8H, m, 4xC$\underline{H}CH_2OAc$, 2xC$H_3C\underline{H}O$, 2xC$\underline{H}OC$), 4.06-4.10(4H, m, 4xCHC$H_a\underline{H}_b OAc$), 4.23-4.31 (4H, m, 4xCHC$H_a\underline{H}_b OAc$), 4.48 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}(O)_2$), 4.74 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}(O)_2$), 4.88-4.93 (2H, m, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xC$\underline{H}$OC), 5.16 (2H, dxd, *J*=9.4 H, *J*=9.4 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 14.1 ($CH_2\underline{C}H_3$), 20.4 (2x$\underline{C}H_3$C=O), 20.5 (6x$\underline{C}H_3$C=O), 20.5 (2x$\underline{C}H_3$C=O), 20.6 (4x$\underline{C}H_3$C=O), 20.6 ($\underline{C}H_2CH_3$), 21.2 (2xCH$\underline{C}H_3$), 25.0 (2x$\underline{C}H_2(CH_2)_2$), 27.0 (2x$\underline{C}H_2CH_2N$), 27.7 (2x$\underline{C}H_2(CH_2)_2$), 29.1 ($\underline{C}H_2CH_2N$),

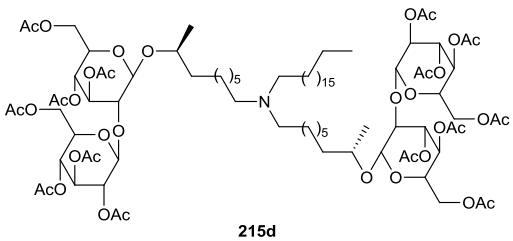


29.7 (2x$\underline{C}H_2(CH_2)_2$), 29.8 (2x$\underline{C}H_2(CH_2)_2$), 36.4 (2x$\underline{C}H_2CHCH_3$), 53.7 ($\underline{C}H_2N$), 54.2 (2x$\underline{C}H_2N$), 61.9 (2x$\underline{C}H_2OAc$), 62.2 (2x$\underline{C}H_2OAc$), 68.2 (2x$\underline{C}HOC$), 68.8 (2x$\underline{C}HOC$), 71.2 (2x$\underline{C}HOC$), 71.6 (2x$\underline{C}HOC$), 71.8 (2x$\underline{C}HOC$), 73.0 (2x$\underline{C}HOC$), 74.6 (2x$\underline{C}HOC$), 77.6 (2x$\underline{C}HOC$), 77.8 (2x$\underline{C}HOC$), 100.3 (2x$\underline{C}H(O)_2$), 101.0 (2x$\underline{C}H(O)_2$), 169.2 (2x$CH_3\underline{C}$=O), 169.3 (2x$CH_3\underline{C}$=O), 169.7 (2x$CH_3\underline{C}$=O), 169.9 (2x$CH_3\underline{C}$=O), 170.2 (2x$CH_3\underline{C}$=O), 170.5 (2x$CH_3\underline{C}$=O), 170.5 (2x$CH_3\underline{C}$=O).

***N*-benzyl,*N*,*N*-bis(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (215c):** (0.38 g, 39%), white sticky solid. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.21 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.25-1.42 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a\underline{H}_bCHCH_3$), 1.44-1.51 (4H, m, 2xC$\underline{H}_2CH_2N$), 1.54-1.63 (2H, m, 2xC$H_a\underline{H}_bCHCH_3$), 1.97 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.07 (6H, s, 2xC$\underline{H}_3$C=O), 2.07 (6H, s, 2xC$\underline{H}_3$C=O), 2.39-2.42 (4H, m, 2xC$\underline{H}_2$N), 3.55 (2H, s, $C_{arom}C\underline{H}_2N$), 3.63-3.74 (8H, m, 4xC$\underline{H}CH_2$OAc, 2xC$H_3C\underline{H}$O, 2xC$\underline{H}$OC), 4.06-4.10 (4H, m, 4xCHC$\underline{H}_a\underline{H}_b$OAc), 4.23-4.30 (4H, m, 4xCHC$H_a\underline{H}_b$OAc), 4.48 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}(O)_2$), 4.73 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}(O)_2$), 4.91 (2H, dxd, *J*=9.1 Hz, *J*=9.1 Hz, 2xC$\underline{H}$OC), 4.93 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2xC$\underline{H}$OC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.13 (2H, dxd, *J*=9.3 Hz, *J*=9.3 Hz, 2xC$\underline{H}$OC), 5.16 (2H, dxd, *J*=9.4 H, *J*=9.4 Hz, 2xC$\underline{H}$OC), 7.19-7.23 (1H, m, C$\underline{H}_{arom}$), 7.27-7.33 (4H, m, 4xC$\underline{H}_{arom}$). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (2x$\underline{C}H_3$C=O), 20.6 (4x$\underline{C}H_3$C=O), 20.6 (2x$\underline{C}H_3$C=O), 20.7 (4x$\underline{C}H_3$C=O), 20.8 (2x$\underline{C}H_3$C=O), 21.2 (2xCH$\underline{C}H_3$), 25.1 (2x$\underline{C}H_2(CH_2)_2$), 27.0 (2x$\underline{C}H_2CH_2N$), 27.6 (2x$\underline{C}H_2(CH_2)_2$), 29.8 (4x$\underline{C}H_2(CH_2)_2$), 36.4 (2x$\underline{C}H_2CHCH_3$), 53.9 (2x$\underline{C}H_2N$), 58.4 ($C_{arom}\underline{C}H_2N$), 62.0 (2x$\underline{C}H_2$OAc), 62.2 (2x$\underline{C}H_2$OAc), 68.2 (2x$\underline{C}$HOC), 68.9 (2x$\underline{C}$HOC), 71.3 (2x$\underline{C}$HOC), 71.7 (2x$\underline{C}$HOC), 71.8 (2x$\underline{C}$HOC), 73.0 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 77.8 (2x$\underline{C}$HOC), 100.4 (2x$\underline{C}H(O)_2$), 101.0 (2x$\underline{C}H(O)_2$), 126.5 ($\underline{C}H_{arom}$), 128.0 (2x$\underline{C}H_{arom}$), 128.8 (2x$\underline{C}H_{arom}$), 140.4 ($\underline{C}_{arom}$), 169.3 (2x$CH_3\underline{C}$=O), 169.4 (2x$CH_3\underline{C}$=O), 169.7 (2x$CH_3\underline{C}$=O), 170.0 (2x$CH_3\underline{C}$=O), 170.3 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O), 170.6 (2x$CH_3\underline{C}$=O).



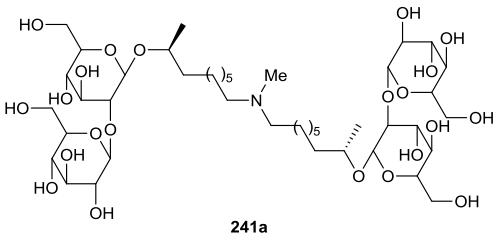
***N*-octadecyl,*N*,*N*-bis(*(S)*-8-*L*-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (215d):** (0.19 g, 18%), white sticky solid. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 0.88 (3H, t, *J*=6.8 Hz, $CH_2C\underline{H}_3$), 1.22 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.24-1.48 (54H, m, 22xC$\underline{H}_2(CH_2)_2$, C$\underline{H}_2CH_3$, 2xC$\underline{H}_a\underline{H}_bCHCH_3$, 3xC$\underline{H}_2CH_2N$), 1.55-1.64 (2H, m, 2xC$H_a\underline{H}_bCHCH_3$), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.01 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (6H, s, 2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (12H, s, 4xC$\underline{H}_3$C=O), 2.37-2.43 (6H, m, 3xC$\underline{H}_2$N), 3.63-3.75



(8H, m, 4xCHCH$_2$OAc, 2xCH$_3$CHO, 2xCHOC), 4.06-4.10 (4H, m, 4xCHCH$_a$H$_b$OAc), 4.23-4.31 (4H, m, 4xCHCH$_a$H$_b$OAc), 4.48 (2H, d, *J*=7.6 Hz, 2xCH(O)$_2$), 4.74 (2H, d, *J*=8.0 Hz, 2xCH(O)$_2$), 4.91 (2H, dxd, *J*=9.0 Hz, *J*=9.0 Hz, 2xCHOC), 4.93 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xCHOC), 5.06 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xCHOC), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xCHOC), 5.16 (2H, dxd, *J*=9.4 H, *J*=9.4 Hz, 2xCHOC). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ_C 14.1 (CH$_2$CH$_3$), 20.4 (2xCH$_3$C=O), 20.5 (2xCH$_3$C=O), 20.5 (2xCH$_3$C=O), 20.5 (2xCH$_3$C=O), 20.7 (4xCH$_3$C=O), 20.7 (2xCH$_3$C=O), 21.2 (2xCHCH$_3$), 22.6 (CH$_2$CH$_3$), 25.0 (2xCH$_2$(CH$_2$)$_2$), 27.0 (3xCH$_2$CH$_2$N), 27.7 (CH$_2$(CH$_2$)$_2$), 27.8 (2xCH$_2$(CH$_2$)$_2$), 29.3 (CH$_2$(CH$_2$)$_2$), 29.6 (CH$_2$(CH$_2$)$_2$), 29.6 (10xCH$_2$(CH$_2$)$_2$), 29.8 (2xCH$_2$(CH$_2$)$_2$), 29.8 (2xCH$_2$(CH$_2$)$_2$), 31.9 (CH$_2$(CH$_2$)$_2$), 36.4 (2xCH$_2$CHCH$_3$), 54.1 (CH$_2$N), 54.2 (2xCH$_2$N), 61.9 (2xCH$_2$OAc), 62.2 (2xCH$_2$OAc), 68.2 (2xCHOC), 68.8 (2xCHOC), 71.2 (2xCHOC), 71.7 (2xCHOC), 71.8 (2xCHOC), 73.0 (2xCHOC), 74.6 (2xCHOC), 77.6 (2xCHOC), 77.8 (2xCHOC), 100.4 (2xCH(O)$_2$), 101.0 (2xCH(O)$_2$), 169.2 (2xCH$_3$C=O), 169.4 (2xCH$_3$C=O), 169.7 (2xCH$_3$C=O), 170.0 (2xCH$_3$C=O), 170.2 (2xCH$_3$C=O), 170.5 (2xCH$_3$C=O), 170.5 (2xCH$_3$C=O).

General procedure for the synthesis of deprotected *N*-alkyl bolaamphiphilic sophorolipids (241): In a 50 mL flask, *N*,*N*'-dialkyl bolaamphiphilic sophorolipid **215** (0.45 mmol, 1 eq) was dissolved in a methanol/water mixture (1:1) and 13 mL Et$_3$N (0.90 mmol, 2 eq) was added. The mixture was stirred for 2 h at reflux temperature and concentrated under reduced pressure to yield pure *N*,*N*'-dialkyl bolaamphiphilic sophorolipid **241**.

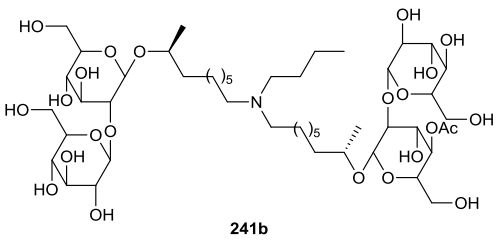
***N*-methyl,*N*,*N*-bis(*(S)*-8-*L*-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl amine (241a):**



241a

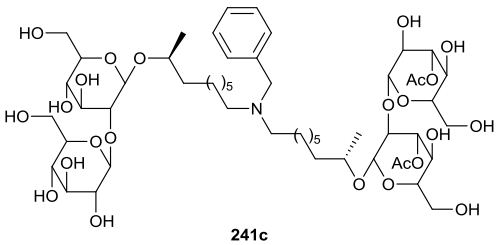
(0.078 g, quant.), white powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.16 (6H, d, *J*=6.1 Hz, 2xCHCH$_3$), 1.18-1.45 (18H, m, 8xCH$_2$(CH$_2$)$_2$, 2xCH$_a$H$_b$CHCH$_3$), 1.47-1.54 (2H, m, 2xCH$_a$H$_b$CHCH$_3$), 1.56-1.63 (4H, m, 2xCH$_2$CH$_2$N), 2.61 (3H, s, CH$_3$N), 2.84-2.88 (4H, m, 2xCH$_2$N), 3.11-3.25 (10H, m, 10xCHOC), 3.30 (2H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, 2xCHOC), 3.36-3.40 (2H, m, 2xCHOC), 3.47 (2H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, 2xCHOC), 3.54-3.59 (4H, m, 4xCHCH$_a$H$_b$OH), 3.70-3.78 (6H, m, 4xCHCH$_a$H$_b$OH, 2xCHOC), 4.35 (2H, d, *J*=7.6 Hz, 2xCH(O)$_2$), 4.58 (2H, d, *J*=7.7 Hz, 2xCH(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.7 (2xCHCH$_3$), 24.3 (2xCH$_2$CH$_2$N), 24.7 (2xCH$_2$(CH$_2$)$_2$), 26.5 (2xCH$_2$(CH$_2$)$_2$), 29.0 (2xCH$_2$(CH$_2$)$_2$), 29.3 (2xCH$_2$(CH$_2$)$_2$), 36.5 (2xCH$_2$CHCH$_3$), 39.3 (CH$_3$N), 56.2 (2xCH$_2$N), 61.4 (2xCH$_2$OH), 61.7 (2xCH$_2$OH), 70.1 (2xCHOC), 70.4 (2xCHOC), 74.6 (2xCHOC), 76.4 (4CHOC), 76.8 (2xCHOC), 76.9 (2xCHOC), 77.6 (2xCHOC), 80.1 (2xCHOC), 101.6 (2xCH(O)$_2$), 103.1 (2xCH(O)$_2$).

***N*-butyl,*N*,*N*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (241b):**


241b

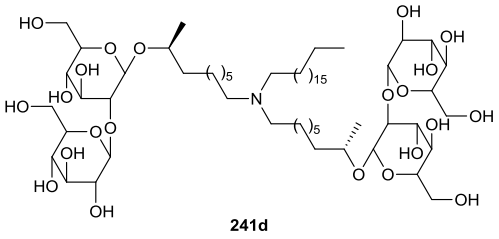
(0.060 g, 92%), white powder. **^{1}H-NMR (400 MHz, MeOD): δ_H** 0.99 (3H, t, *J*=7.3 Hz, $CH_2C\underline{H}_3$), 1.25 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.29-1.53 (20H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$, C$\underline{H}_2$CH$_3$), 1.56-1.67 (8H, m, 2xCH$_a\underline{H}_b$CHCH$_3$, 6xC$\underline{H}_2$CH$_2$N), 2.88-2.92 (6H, m, 3xC$\underline{H}_2$N), 3.21-3.35 (10H, m, 10xC$\underline{H}$OC), 3.39 (2H, dxd, *J*=8.9 Hz, *J*=8.9 Hz, 2xC$\underline{H}$OC), 3.47 (2H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, 2xC$\underline{H}$OC), 3.56 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.63-3.68 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.80-3.87 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.45 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}$(O)$_2$), 4.67 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 12.8 ($CH_2\underline{C}H_3$), 19.9 ($\underline{C}H_2CH_3$), 20.6 (2xCH$\underline{C}$H$_3$), 24.3 (2x$\underline{C}$H$_2$CH$_2$N), 24.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.2 ($\underline{C}$H$_2$CH$_2$N), 26.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.4 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 52.6 ($\underline{C}$H$_2$N), 53.0 (2x$\underline{C}$H$_2$N), 61.4 (2x$\underline{C}$H$_2$OH), 61.7 (2x$\underline{C}$H$_2$OH), 70.1 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.4 (4x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.6 (2x$\underline{C}$HOC), 80.2 (2x$\underline{C}$HOC), 101.5 (2x$\underline{C}$H(O)$_2$), 103.1 (2x$\underline{C}$H(O)$_2$).

***N*-benzyl,*N*,*N*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl amine (241c):**


241c

(0.10 g, 80%), white powder. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.27 (6H, d, *J*=6.1 Hz, 2xCHC$\underline{H}_3$), 1.29-1.50 (18H, m, 8xC$\underline{H}_2$(CH$_2$)$_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.53-1.65 (6H, m, 2xCH$_a\underline{H}_b$CHCH$_3$, 4xC$\underline{H}_2$CH$_2$N), 2.57-2.61 (4H, m, 2xC$\underline{H}_2$N), 3.24-3.37 (10H, m, 10xC$\underline{H}$OC), 3.41 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.49 (2H, dxd, *J*=8.4 Hz, *J*=8.4 Hz, 2xC$\underline{H}$OC), 3.59 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.65-3.71 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.77 (2H, s, C$_{arom}$C$\underline{H}_2$N), 3.81-3.89 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.47 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}$(O)$_2$), 4.67 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}$(O)$_2$), 7.30-7.39 (5H, m, 5xCH$_{arom}$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 20.6 (2xCH$\underline{C}$H$_3$), 24.8 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 25.5 (2x$\underline{C}$H$_2$CH$_2$N), 27.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.2 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.4 (2x$\underline{C}$H$_2$CHCH$_3$), 53.2 (2x$\underline{C}$H$_2$N), 57.8 (C$_{arom}\underline{C}$H$_2$N), 61.4 (2x$\underline{C}$H$_2$OH), 61.7 (2x$\underline{C}$H$_2$OH), 70.1 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.5 (2x$\underline{C}$HOC), 76.3 (2x$\underline{C}$HOC), 76.4 (2x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.5 (2x$\underline{C}$HOC), 80.4 (2x$\underline{C}$HOC), 101.4 (2x$\underline{C}$H(O)$_2$), 103.2 (2x$\underline{C}$H(O)$_2$), 127.4 ($\underline{C}$H$_{arom}$), 128.1 (2x$\underline{C}$H$_{arom}$), 129.5 (2x$\underline{C}$H$_{arom}$), 136.7 ($\underline{C}_{arom}$).

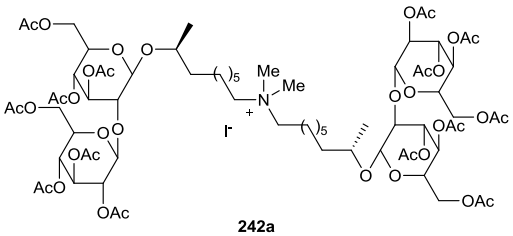
***N*-octadecyl,*N*,*N*-bis(*(S)*-8-*L*-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl amine**


241d

(241d): (0.088 g, 99%), white powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.90 (3H, t, *J*=6.8 Hz, $CH_2C\underline{H}_3$), 1.26 (6H, d, *J*=6.2 Hz, 2x$CHC\underline{H}_3$), 1.28-1.54 (48H, m, 22x$C\underline{H}_2(CH_2)_2$, 2x$C\underline{H}_aH_bCHCH_3$, $C\underline{H}_2CH_3$), 1.56-1.68 (8H, m, 3x$CH_a\underline{H}_bCHCH_3$, 4x$C\underline{H}_2CH_2N$), 2.90-2.96 (6H, m, 3x$C\underline{H}_2N$), 3.21-3.34 (10H, m, 10x$C\underline{H}OC$), 3.40 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2x$C\underline{H}OC$), 3.45-3.50 (2H, m, 2x$C\underline{H}OC$), 3.57 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2x$C\underline{H}OC$), 3.64-3.69 (4H, m, 4x$CHC\underline{H}_aH_bOH$), 3.80-3.87 (6H, m, 4x$CHCH_a\underline{H}_bOH$, 2x$C\underline{H}OC$), 4.45 (2H, d, *J*=7.7 Hz, 2x$C\underline{H}(O)_2$), 4.67 (2H, d, *J*=7.7 Hz, 2x$C\underline{H}(O)_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 13.1 ($CH_2\underline{C}H_3$), 20.7 (2x$CH\underline{C}H_3$), 22.4 ($\underline{C}H_2CH_3$), 24.0 ($\underline{C}H_2CH_2N$), 24.1 (2x$\underline{C}H_2CH_2N$), 24.7 (2x$\underline{C}H_2(CH_2)_2$), 26.6 ($\underline{C}H_2(CH_2)_2$), 26.7 (2x$\underline{C}H_2(CH_2)_2$), 29.0 ($\underline{C}H_2(CH_2)_2$), 29.1 (2x$\underline{C}H_2(CH_2)_2$), 29.2 ($\underline{C}H_2(CH_2)_2$), 29.3 ($\underline{C}H_2(CH_2)_2$), 29.4 (11x$\underline{C}H_2(CH_2)_2$), 31.7 ($\underline{C}H_2(CH_2)_2$), 36.5 (2x$\underline{C}H_2CHCH_3$), 52.8 ($\underline{C}H_2N$), 52.9 (2x$\underline{C}H_2N$), 61.4 (2x$\underline{C}H_2OH$), 61.7 (2x$\underline{C}H_2OH$), 70.1 (2x$\underline{C}HOC$), 70.4 (2x$\underline{C}HOC$), 74.6 (2x$\underline{C}HOC$), 76.4 (4x$\underline{C}HOC$), 76.8 (2x$\underline{C}HOC$), 76.9 (2x$\underline{C}HOC$), 77.6 (2x$\underline{C}HOC$), 80.2 (2x$\underline{C}HOC$), 101.5 (2x$\underline{C}H(O)_2$), 103.1 (2x$\underline{C}H(O)_2$).

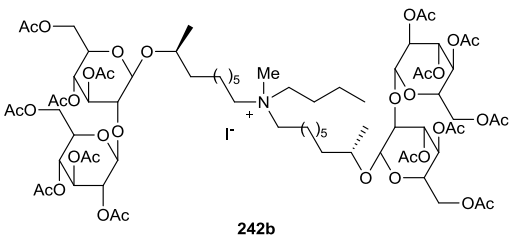
General procedure for the synthesis of peracetylated monocationic bolaamphiphilic sophorolipids (242): In a 10 mL flame dried pressure resistant vial, peracetylated *N*,*N*'dialkyl bolaamphiphilic sophorolipid **215** was dissolved in dry acetonitrile. The solution was cooled down to 0 °C and the alkyl iodide (5 eq) was added. The vial was closed and heated to 80 °C for 18 hours. The reaction mixture was concentrated under reduced pressure to yield the peracetylated quaternary ammonium bolaamphiphilic sophorolipid **242**.

***N*,*N*-dimethyl,*N*,*N*-bis(*(S)*-8-*L*-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (242a):** (0.16 g, 94%), yellow powder. **^{1}H-NMR (400 MHz, $CDCl_3$):** δ_H 1.22 (6H, d, *J*=6.2 Hz, 2x$CHC\underline{H}_3$), 1.26-1.49 (18H, m, 8x$C\underline{H}_2(CH_2)_2$, 2x$C\underline{H}_aH_bCHCH_3$), 1.50-1.64 (2H, m, 2x$CH_a\underline{H}_bCHCH_3$), 1.75-1.85 (4H, m, 2x$C\underline{H}_2CH_2N$), 1.99 (6H, s, 2x$C\underline{H}_3C$=O), 2.00 (6H, s, 2x$C\underline{H}_3C$=O), 2.03 (6H, s, 2x$C\underline{H}_3C$=O), 2.03 (6H, s, 2x$C\underline{H}_3C$=O), 2.06 (6H, s, 2x$C\underline{H}_3C$=O), 2.08 (6H, s, 2x$C\underline{H}_3C$=O), 2.09 (6H, s, 2x$C\underline{H}_3C$=O), 3.34 (6H, s, 2x$C\underline{H}_3N$), 3.48-3.52 (4H, m, 2x$C\underline{H}_2N$), 3.66-3.77 (8H, m, 4x$C\underline{H}CH_2OAc$, 2x$CH_3C\underline{H}O$, 2x$C\underline{H}OC$), 4.05-4.10 (4H, m, 4x$CHC\underline{H}_aH_bOAc$), 4.23-4.27 (4H, m, 4x$CHCH_a\underline{H}_bOAc$), 4.49 (2H, d, *J*=7.6 Hz, 2x$C\underline{H}(O)_2$), 4.72 (2H, d, *J*=8.0 Hz, 2x$C\underline{H}(O)_2$), 4.86 (2H, dxd, *J*=9.5 Hz, *J*=8.2 Hz, 2x$C\underline{H}OC$), 4.92 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2x$C\underline{H}OC$), 5.00 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2x$C\underline{H}OC$), 5.12 (2H, dxd, *J*=9.9 Hz, *J*=9.9 Hz, 2x$C\underline{H}OC$), 5.17 (2H, dxd, *J*=9.6 H, *J*=9.6


242a

Hz, 2xCHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 20.5 (2xCH_3C=O), 20.6 (2xCH_3C=O), 20.8 (6xCH_3C=O), 20.9 (4xCH_3C=O), 21.6 (2xCHCH_3), 22.8 (2xCH_2CH_2N), 24.7 (2x$CH_2(CH_2)_2$), 26.3 (2x$CH_2(CH_2)_2$), 29.4 (4x$CH_2(CH_2)_2$), 36.6 (2xCH_2CHCH_3), 51.6 (2xCH_3N), 62.3 (2xCH_2OAc), 62.4 (2xCH_2OAc), 64.8 (2xCH_2N), 68.6 (2xCHOC), 68.9 (2xCHOC), 71.3 (2xCHOC), 71.5 (2xCHOC), 71.7 (2xCHOC), 73.0 (2xCHOC), 74.8 (2xCHOC), 77.8 (2xCHOC), 78.0 (2xCHOC), 100.5 (2x$CH(O)_2$), 101.3 (2x$CH(O)_2$), 169.7 (2xCH_3C=O), 169.7 (2xCH_3C=O), 169.8 (2xCH_3C=O), 170.0 (4xCH_3C=O), 170.6 (4xCH_3C=O).

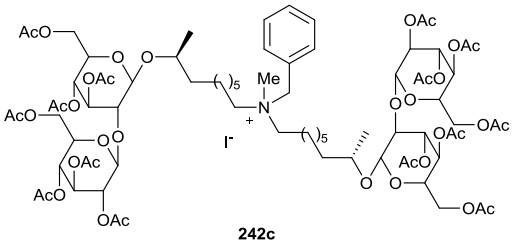
***N*-butyl,*N*-methyl,*N*,*N*-bis(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (242b):** (0.16 g, 95%), light yellow powder. **^{1}H-NMR**



242b

(400 MHz, $CDCl_3$): δ_H 1.02 (3H, t, *J*=7.3 Hz, CH_2CH_3), 1.22 (6H, d, *J*=6.2 Hz, 2xCHCH_3), 1.26-1.50 (20H, m, 8x$CH_2(CH_2)_2$, 2x$CH_aH_bCHCH_3$, CH_2CH_3), 1.52-1.63 (2H, m, 2x$CH_aH_bCHCH_3$), 1.71-1.81 (6H, m, 3xCH_2CH_2N), 1.99 (6H, s, 2xCH_3C=O), 2.00 (6H, s, 2xCH_3C=O), 2.03 (6H, s, 2xCH_3C=O), 2.04 (6H, s, 2xCH_3C=O), 2.06 (6H, s, 2xCH_3C=O), 2.08 (6H, s, 2xCH_3C=O), 2.09 (6H, s, 2xCH_3C=O), 3.27 (3H, s, CH_3N), 3.40-3.51 (6H, m 3xCH_2N), 3.66-3.77 (8H, m, 4xCHCH_2OAc, 2xCH_3CHO, 2xCHOC), 4.05-4.10 (4H, m, 4xCHCH_aH_bOAc), 4.23-4.27 (4H, m, 4xCHCH_aH_bOAc), 4.49 (2H, d, *J*=7.7 Hz, 2xCH$(O)_2$), 4.73 (2H, d, *J*=8.0 Hz, 2xCH$(O)_2$), 4.86 (2H, dxd, *J*=9.4 Hz, *J*=8.2 Hz, 2xCHOC), 4.92 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xCHOC), 5.00 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xCHOC), 5.13 (2H, dxd, *J*=9.4 Hz, *J*=9.4 Hz, 2xCHOC), 5.17 (2H, dxd, *J*=9.4 H, *J*=9.4 Hz, 2xCHOC). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ_C 13.8 (CH_2CH_3), 19.7 (CH_2CH_3), 20.5 (2xCH_3C=O), 20.6 (2xCH_3C=O), 20.7 (4xCH_3C=O), 20.7 (2xCH_3C=O), 20.8 (4xCH_3C=O), 21.5 (2xCHCH_3), 22.5 (2xCH_2CH_2N), 24.4 (CH_2CH_2N), 24.7 (2x$CH_2(CH_2)_2$), 26.5 (2x$CH_2(CH_2)_2$), 29.4 (4x$CH_2(CH_2)_2$), 36.5 (2xCH_2CHCH_3), 49.4 (CH_3N), 61.7 (CH_2N), 61.9 (2xCH_2N), 62.2 (2xCH_2OAc), 62.4 (2xCH_2OAc), 68.5 (2xCHOC), 68.9 (2xCHOC), 71.3 (2xCHOC), 71.5 (2xCHOC), 71.7 (2xCHOC), 73.0 (2xCHOC), 74.8 (2xCHOC), 77.8 (2xCHOC), 77.9 (2xCHOC), 100.5 (2x$CH(O)_2$), 101.2 (2x$CH(O)_2$), 169.6 (4xCH_3C=O), 169.8 (2xCH_3C=O), 170.0 (2xCH_3C=O), 170.1 (2xCH_3C=O), 170.6 (2xCH_3C=O), 170.6 (2xCH_3C=O).

***N*-benzyl,*N*-methyl,*N*,*N*-bis(*(S)*-8-[(2”,3’,3”,4’,4”,6’,6”-heptaacetoxy-2’-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (242c):** (0.26 g, 96%), light yellow powder. **^{1}H-NMR**

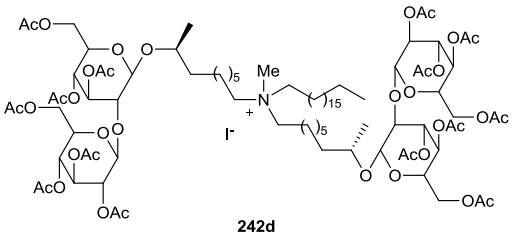


242c

(400 MHz, $CDCl_3$): δ_H 1.22 (6H, d, *J*=6.2 Hz, 2xCHCH_3), 1.25-1.47 (18H, m, 8x$CH_2(CH_2)_2$, 2x$CH_aH_bCHCH_3$), 1.51-1.61 (2H, m, 2x$CH_aH_bCHCH_3$), 1.81-1.93 (4H, m, 2xCH_2CH_2N), 1.95 (6H, s, 2xCH_3C=O), 2.00 (6H, s, 2xCH_3C=O), 2.01 (6H, s, 2xCH_3C=O), 2.02 (6H, s,

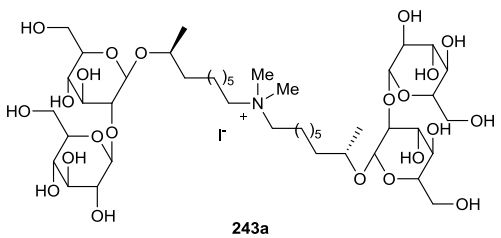
2xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.09 (6H, s, 2xC$\underline{H}_3$C=O), 3.23 (3H, s, C$\underline{H}_3$N), 3.36-3.47 (4H, m, 2xC$\underline{H}_2$N), 3.65-3.76 (8H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC), 4.06-4.11 (4H, m, 4xCHC$\underline{H}_a$H$_b$OAc), 4.22-4.27 (4H, m, 4xCHCH$_a$$\underline{H}_b$OAc), 4.48 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.72 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}$(O)$_2$), 4.85-4.90 (2H, m, 2xC$\underline{H}$OC), 4.90 (2H, s, C$_{arom}$C$\underline{H}_2$N), 4.93 (2H, dxd, *J*=9.9 Hz, *J*=9.9 Hz, 2xC$\underline{H}$OC), 5.01 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.12 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.17 (2H, dxd, *J*=9.5 H, *J*=9.5 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 20.5 (2x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.7 (2x$\underline{C}$H$_3$C=O), 20.8 (4x$\underline{C}$H$_3$C=O), 21.5 (2xCH$\underline{C}$H$_3$), 22.6 (2x$\underline{C}$H$_2$CH$_2$N), 24.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.4 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.4 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.5 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 47.8 ($\underline{C}$H$_3$N), 60.7 (2x$\underline{C}$H$_2$N), 62.2 (2x$\underline{C}$H$_2$OAc), 62.3 (2x$\underline{C}$H$_2$OAc), 65.1 (C$_{arom}$$\underline{C}H_2$N), 68.5 (2x$\underline{C}$HOC), 68.8 (2x$\underline{C}$HOC), 71.3 (2x$\underline{C}$HOC), 71.5 (2x$\underline{C}$HOC), 71.7 (2x$\underline{C}$HOC), 72.9 (2x$\underline{C}$HOC), 74.7 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 77.9 (2x$\underline{C}$HOC), 100.4 (2x$\underline{C}$H(O)$_2$), 101.2 (2x$\underline{C}$H(O)$_2$), 127.0 ($\underline{C}_{arom}$), 129.3 (2x$\underline{C}H_{arom}$), 130.8 ($\underline{C}H_{arom}$), 133.1 (2x$\underline{C}H_{arom}$), 169.6 (4xCH$_3\underline{C}$=O), 169.7 (2xCH$_3\underline{C}$=O), 170.0 (2xCH$_3\underline{C}$=O), 170.0 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O), 170.6 (2xCH$_3\underline{C}$=O).

***N*-methyl,*N*-octadecyl,*N*,*N*-bis(*(S)*-8-[(2",3',3",4',4",6',6"-heptaacetoxy-2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (242d):** (0.18 g, 97%), light yellow powder. **^{1}H-NMR (400 MHz, CDCl$_3$): δ_H** 0.88 (3H, t, *J*=6.8 Hz, CH$_2$C$\underline{H}_3$), 1.22 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.24-1.47 (48H, m, 22xC$\underline{H}_2$(CH$_2$)$_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$, C$\underline{H}_2$CH$_3$), 1.52-1.62 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.71-1.81 (6H, m, 3xC$\underline{H}_2$CH$_2$N), 1.99 (6H, s, 2xC$\underline{H}_3$C=O), 2.00 (6H, s, 2xC$\underline{H}_3$C=O), 2.03 (12H, s, 4xC$\underline{H}_3$C=O), 2.06 (6H, s, 2xC$\underline{H}_3$C=O), 2.08 (6H, s, 2xC$\underline{H}_3$C=O), 2.09 (6H, s, 2xC$\underline{H}_3$C=O), 3.31 (3H, s, C$\underline{H}_3$N), 3.41-3.52 (6H, m, 3xC$\underline{H}_2$N), 3.66-3.75 (8H, m, 4xC$\underline{H}$CH$_2$OAc, 2xCH$_3$C$\underline{H}$O, 2xC$\underline{H}$OC), 4.05-4.11 (4H, m, 4xCHC$\underline{H}_a$H$_b$OAc), 4.23-4.28 (4H, m, 4xCHCH$_a$$\underline{H}_b$OAc), 4.49 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.72 (2H, d, *J*=8.0 Hz, 2xC$\underline{H}$(O)$_2$), 4.87 (2H, dxd, *J*=9.5 Hz, *J*=8.1 Hz, 2xC$\underline{H}$OC), 4.92 (2H, dxd, *J*=9.7 Hz, *J*=9.7 Hz, 2xC$\underline{H}$OC), 5.01 (2H, dxd, *J*=9.6 Hz, *J*=9.6 Hz, 2xC$\underline{H}$OC), 5.12 (2H, dxd, *J*=9.5 Hz, *J*=9.5 Hz, 2xC$\underline{H}$OC), 5.18 (2H, dxd, *J*=9.5 H, *J*=9.5 Hz, 2xC$\underline{H}$OC). **^{13}C-NMR (100 MHz, CDCl$_3$): δ_C** 14.0 (CH$_2\underline{C}$H$_3$), 20.4 (2x$\underline{C}$H$_3$C=O), 20.5 (2x$\underline{C}$H$_3$C=O), 20.6 (4x$\underline{C}$H$_3$C=O), 20.6 (2x$\underline{C}$H$_3$C=O), 20.7 (2x$\underline{C}$H$_3$C=O), 20.7 (2x$\underline{C}$H$_3$C=O), 21.4 (2xCH$\underline{C}$H$_3$), 22.4 (2x$\underline{C}$H$_2$CH$_2$N), 22.5 ($\underline{C}$H$_2$CH$_2$N), 22.6 ($\underline{C}$H$_2$CH$_3$), 24.6 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.3 ($\underline{C}$H$_2$(CH$_2$)$_2$), 26.3 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.2-29.6 (16x$\underline{C}$H$_2$(CH$_2$)$_2$), 31.8 ($\underline{C}$H$_2$(CH$_2$)$_2$), 36.4 (2x$\underline{C}$H$_2$CHCH$_3$), 49.0 ($\underline{C}$H$_3$N), 61.7 (3x$\underline{C}$H$_2$N), 62.1 (2x$\underline{C}$H$_2$OAc), 62.2 (2x$\underline{C}$H$_2$OAc), 68.4 (2x$\underline{C}$HOC), 68.8 (2x$\underline{C}$HOC), 71.2 (2x$\underline{C}$HOC), 71.4 (2x$\underline{C}$HOC), 71.6 (2x$\underline{C}$HOC), 72.9 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 77.8 (2x$\underline{C}$HOC), 100.4 (2x$\underline{C}$H(O)$_2$), 101.1 (2x$\underline{C}$H(O)$_2$), 169.5 (4xCH$_3\underline{C}$=O), 169.6 (2xCH$_3\underline{C}$=O), 169.9 (4xCH$_3\underline{C}$=O), 170.4 (2xCH$_3\underline{C}$=O), 170.5 (2xCH$_3\underline{C}$=O).

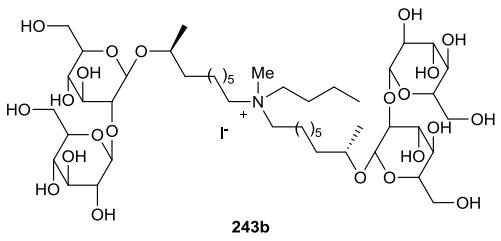


General procedure for the synthesis of deprotected monocationic bolaamphiphilic sophorolipids (243): In a 50 mL flask, peracetylated monocationic bolaamphiphilic sophorolipid **242** (0.45 mmol, 1 eq) was dissolved in a methanol/water mixture (1:1) and 13 mL Et_3N (0.90 mmol, 2 eq) was added. The mixture was stirred for 2 h at reflux temperature and concentrated under reduced pressure to yield pure deprotected monocationic bolaamphiphilic sophorolipid **243**.

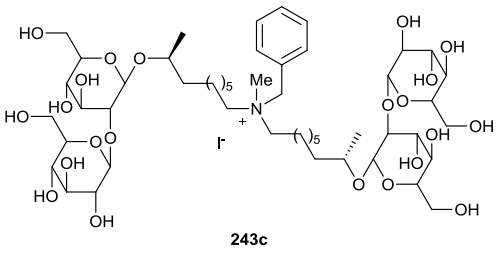
***N,N*-dimethyl,*N,N*-bis(*(S)*-8-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (243a):** (0.069 g, 80%), yellow powder. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.31 (6H, d, *J*=6.1 Hz, 2xCHC$\underline{H}_3$), 1.34-1.59 (18H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$), 1.60-1.70 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.80-1.88 (4H, m, 2xC$\underline{H}_2$CH$_2$N), 3.15 (6H, s, 2xC$\underline{H}_3$N), 3.26-3.42 (14H, m, 10xC$\underline{H}$OC, 2xC$\underline{H}_2$N), 3.45 (2H, dxd, *J*=8.3 Hz, *J*=8.3 Hz, 2xC$\underline{H}$OC), 3.51-3.56 (2H, m, 2xC$\underline{H}$OC), 3.63 (2H, dxd, *J*=8.5 Hz, *J*=8.5 Hz, 2xC$\underline{H}$OC), 3.69-3.73 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.86-3.93 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.51 (2H, d, *J*=7.7 Hz, 2xC$\underline{H}$(O)$_2$), 4.74 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 20.7 (2xCH$\underline{C}$H$_3$), 22.3 (2x$\underline{C}$H$_2$CH$_2$N), 24.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 50.0 (2x$\underline{C}$H$_3$N),61.4 (2x$\underline{C}$H$_2$OH), 61.6 (2x$\underline{C}$H$_2$OH), 64.2 (2x$\underline{C}$H$_2$N), 70.2 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.4 (4x$\underline{C}$HOC), 76.8 (2x$\underline{C}$HOC), 77.0 (2x$\underline{C}$HOC), 77.7 (2x$\underline{C}$HOC), 80.0 (2x$\underline{C}$HOC), 101.6 (2x$\underline{C}$H(O)$_2$), 103.0 (2x$\underline{C}$H(O)$_2$).



***N*-butyl,*N*-methyl,*N,N*-bis(*(S)*-8-[(2'-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (243b):** (0.082 g, 96%), yellow powder. **^{1}H-NMR (400 MHz, MeOD): δ_H** 1.06 (3H, t, *J*=7.4 Hz, CH$_2$C$\underline{H}_3$), 1.28 (6H, d, *J*=6.2 Hz, 2xCHC$\underline{H}_3$), 1.32-1.56 (20H, m, 8xC$\underline{H}_2(CH_2)_2$, 2xC$\underline{H}_a$H$_b$CHCH$_3$, C$\underline{H}_2$CH$_3$), 1.58-1.67 (2H, m, 2xCH$_a\underline{H}_b$CHCH$_3$), 1.70-1.80 (6H, m, 3xC$\underline{H}_2$CH$_2$N), 3.06 (3H, s, C$\underline{H}_3$N), 3.24 (2H, dxd, *J*=9.3 Hz, *J*=7.8 Hz, 2xC$\underline{H}$OC), 3.25-3.35 (14H, m, 8xC$\underline{H}$OC, 3xC$\underline{H}_2$N), 3.41 (2H, dxd, *J*=8.8 Hz, *J*=8.8 Hz, 2xC$\underline{H}$OC), 3.50 (2H, dxd, *J*=9.1 Hz, *J*=7.8 Hz, 2xC$\underline{H}$OC), 3.58 (2H, dxd, *J*=8.7 Hz, *J*=8.7 Hz, 2xC$\underline{H}$OC), 3.66-3.70 (4H, m, 4xCHC$\underline{H}_a$H$_b$OH), 3.82-3.90 (6H, m, 4xCHCH$_a\underline{H}_b$OH, 2xC$\underline{H}$OC), 4.47 (2H, d, *J*=7.6 Hz, 2xC$\underline{H}$(O)$_2$), 4.70 (2H, d, *J*=7.8 Hz, 2xC$\underline{H}$(O)$_2$). **^{13}C-NMR (100 MHz, MeOD): δ_C** 12.7 (CH$_2\underline{C}$H$_3$), 19.4 ($\underline{C}$H$_2$CH$_3$), 20.7 (2xCH$\underline{C}$H$_3$), 22.0 (2x$\underline{C}$H$_2$CH$_2$N), 23.9 ($\underline{C}$H$_2$CH$_2$N), 24.7 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 26.1 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.0 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 29.3 (2x$\underline{C}$H$_2$(CH$_2$)$_2$), 36.5 (2x$\underline{C}$H$_2$CHCH$_3$), 47.6 ($\underline{C}$H$_3$N), 61.2 ($\underline{C}$H$_2$N), 61.4 (2x$\underline{C}$H$_2$OH), 61.5 (2x$\underline{C}$H$_2$N), 61.6 (2x$\underline{C}$H$_2$OH), 70.2 (2x$\underline{C}$HOC), 70.4 (2x$\underline{C}$HOC), 74.6 (2x$\underline{C}$HOC), 76.3 (4x$\underline{C}$HOC), 76.8 (2x$\underline{C}$HOC), 76.9 (2x$\underline{C}$HOC), 77.6 (2x$\underline{C}$HOC), 80.1 (2x$\underline{C}$HOC), 101.5 (2x$\underline{C}$H(O)$_2$), 103.1 (2x$\underline{C}$H(O)$_2$).

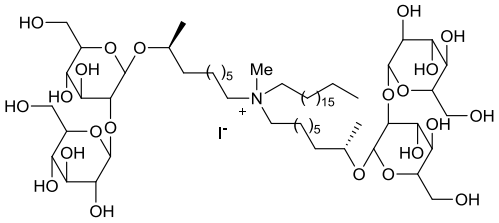


***N*-benzyl,*N*-methyl,*N*,*N*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (243c):** (0.14 g, 96%), yellow powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 1.33 (6H, d, *J*=6.1 Hz, 2xCHC<u>H</u>$_3$), 1.37-1.61 (18H, m, 8xC<u>H</u>$_2$(CH$_2$)$_2$, 2xC<u>H</u>$_a$H$_b$CHCH$_3$), 1.64-1.73 (2H, m, 2xCH$_a$<u>H</u>$_b$CHCH$_3$), 1.87-2.02 (4H, m, 2xC<u>H</u>$_2$CH$_2$N), 3.08 (<u>C</u>H$_3$N), 3.29-3.44 (14H, m, 10xC<u>H</u>OC, 2x<u>C</u>H$_2$N), 3.49 (2H, dxd, *J*=8.6 Hz, *J*=8.6 Hz, 2xC<u>H</u>OC), 3.56 (2H, dxd, *J*=8.4 Hz, *J*=8.4 Hz, 2xC<u>H</u>OC), 3.66 (2H, dxd, *J*=8.3 Hz, *J*=8.3 Hz, 2xC<u>H</u>OC), 3.71-3.77 (4H, m, 4xCHC<u>H</u>$_a$H$_b$OH), 3.87-3.95 (6H, m, 4xCHCH$_a$<u>H</u>$_b$OH, 2xC<u>H</u>OC), 4.54 (2H, d, *J*=7.7 Hz, 2xC<u>H</u>(O)$_2$), 4.67 (2H, s, C$_{arom}$C<u>H</u>$_2$N), 4.75 (2H, d, *J*=7.7 Hz, 2xC<u>H</u>(O)$_2$), 7.59-7.69 (5H, m, 5xC<u>H</u>$_{arom}$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 20.7 (2xCH<u>C</u>H$_3$), 22.2 (2x<u>C</u>H$_2$CH$_2$N), 24.7 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 26.1 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.0 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.3 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 36.5 (2x<u>C</u>H$_2$CHCH$_3$), 47.2 (<u>C</u>H$_3$N), 61.0 (2x<u>C</u>H$_2$N), 61.4 (2x<u>C</u>H$_2$OH), 61.7 (2x<u>C</u>H$_2$OH), 65.3 (<u>C</u>H$_2$N), 70.2 (2x<u>C</u>HOC), 70.4 (2x<u>C</u>HOC), 74.6 (2x<u>C</u>HOC), 76.3 (4x<u>C</u>HOC), 76.8 (2x<u>C</u>HOC), 76.9 (2x<u>C</u>HOC), 77.6 (2x<u>C</u>HOC), 80.1 (2x<u>C</u>HOC), 101.5 (2x<u>C</u>H(O)$_2$), 103.1 (2x<u>C</u>H(O)$_2$), 127.6 (<u>C</u>$_{arom}$), 129.0 (2x<u>C</u>H$_{arom}$), 130.5 (<u>C</u>H$_{arom}$), 132.8 (2x<u>C</u>H$_{arom}$).



243c

***N*-methyl,*N*-octadecyl,*N*,*N*-bis(*(S)*-8-[(2'-*O*-*β*-*D*-glucopyranosyl-*β*-*D*-glucopyranosyl)-oxy])nonyl ammonium iodide (243d):** (0.099 g, 95%), yellow powder. **^{1}H-NMR (400 MHz, MeOD):** δ_H 0.95 (3H, t, *J*=6.7 Hz, CH$_2$C<u>H</u>$_3$), 1.31 (6H, d, *J*=6.2 Hz, 2xCHC<u>H</u>$_3$), 1.33-1.59 (48H, m, 22xC<u>H</u>$_2$(CH$_2$)$_2$, 2xC<u>H</u>$_a$H$_b$CHCH$_3$, C<u>H</u>$_2$CH$_3$), 1.61-1.70 (2H, m, 2xCH$_a$<u>H</u>$_b$CHCH$_3$), 1.75-1.84 (6H, m, 3xC<u>H</u>$_2$CH$_2$N), 3.10 (<u>C</u>H$_3$N), 3.26-3.40 (22H, m, 10xC<u>H</u>OC, 6x<u>C</u>H$_2$N), 3.45 (2H, dxd, *J*=8.4 Hz, *J*=8.4 Hz, 2xC<u>H</u>OC), 3.53 (2H, dxd, *J*=8.3 Hz, *J*=8.3 Hz, 2xC<u>H</u>OC), 3.63 (2H, dxd, *J*=8.5 Hz, *J*=8.5 Hz, 2xC<u>H</u>OC), 3.70-3.73 (4H, m, 4xCHC<u>H</u>$_a$H$_b$OH), 3.85-3.93 (6H, m, 4xCHCH$_a$<u>H</u>$_b$OH, 2xC<u>H</u>OC), 4.51 (2H, d, *J*=7.6 Hz, 2xC<u>H</u>(O)$_2$), 4.73 (2H, d, *J*=7.8 Hz, 2xC<u>H</u>(O)$_2$). **^{13}C-NMR (100 MHz, MeOD):** δ_C 13.1 (CH$_2$<u>C</u>H$_3$), 20.7 (2xCH<u>C</u>H$_3$), 21.9 (<u>C</u>H$_2$CH$_2$N), 22.0 (2x<u>C</u>H$_2$CH$_2$N), 22.4 (<u>C</u>H$_2$CH$_3$), 24.7 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 26.0 (<u>C</u>H$_2$(CH$_2$)$_2$), 26.1 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 28.9 (<u>C</u>H$_2$(CH$_2$)$_2$), 29.0 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.1 (2x<u>C</u>H$_2$(CH$_2$)$_2$), 29.2 (<u>C</u>H$_2$(CH$_2$)$_2$), 29.3 (<u>C</u>H$_2$(CH$_2$)$_2$), 29.4 (9x<u>C</u>H$_2$(CH$_2$)$_2$), 31.7 (<u>C</u>H$_2$(CH$_2$)$_2$), 36.5 (2x<u>C</u>H$_2$CHCH$_3$), 47.6 (<u>C</u>H$_3$N), 61.4 (2x<u>C</u>H$_2$OH), 61.5 (3x<u>C</u>H$_2$N), 61.7 (2x<u>C</u>H$_2$OH), 70.2 (2x<u>C</u>HOC), 70.4 (2x<u>C</u>HOC), 74.6 (2x<u>C</u>HOC), 76.4 (4x<u>C</u>HOC), 76.8 (2x<u>C</u>HOC), 76.9 (2x<u>C</u>HOC), 77.6 (2x<u>C</u>HOC), 80.0 (2x<u>C</u>HOC), 101.5 (2x<u>C</u>H(O)$_2$), 103.1 (2x<u>C</u>H(O)$_2$).

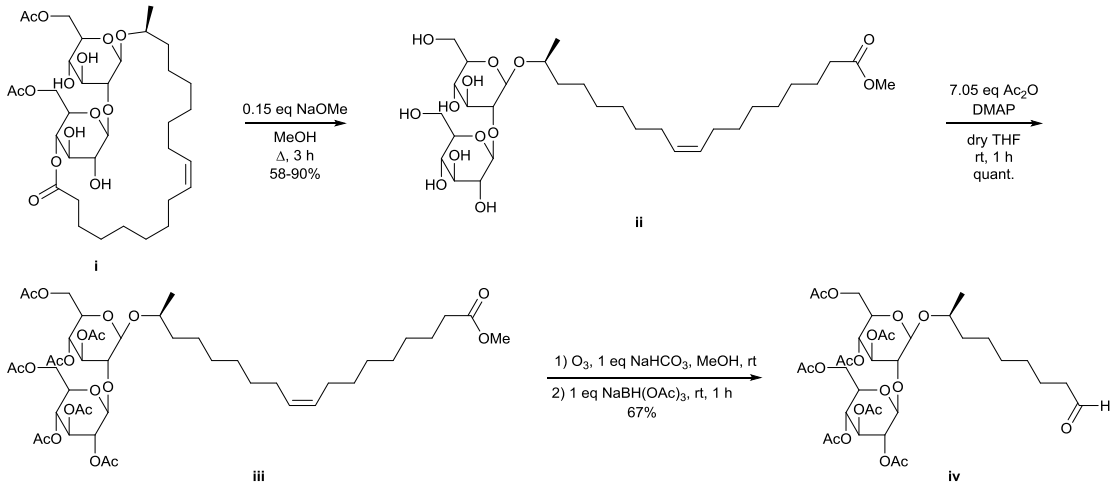


243d

5. Summary

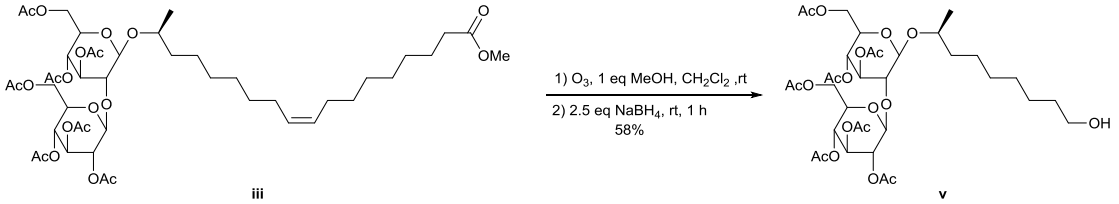
Sophorolipids, a class of glycolipid biosurfactants, are promising renewable-based building blocks for chemical derivatization. They are produced by micro-organisms through fermentation, with the yeast *Starmerella bombicola* being the preferred producing organism. Sophorolipids meet all the criteria to be successful renewable building blocks for chemical derivatization since they comprise a complex structure and can be produced in high quantities and quality. An output of around 400 g/L can be obtained at an estimated production price of 2 to 5 €/kg. *Via* fermentation, multiple sophorolipid derivatives can be produced and selective production of one single sophorolipid derivative can be obtained with genetically modified *S. bombicola* strains. Natural sophorolipids already possess interesting biological and physico-chemical properties. They feature anti-cancer, antimicrobial, dermatological, immunoregulatory, spermicidal and antiviral activities. They also possess self-assembly properties, with a high variety in the type of nanostructures formed for different sophorolipid derivatives.

In this work the major sophorolipid fermentation product, i.e. the diacetylated sophorolipid lactone, was used for the synthesis of a wide range of innovative sophorolipid derivatives. The first goal was to synthesize short-chained intermediate sophorolipid derivatives, in particular a sophorolipid aldehyde, *via* an ozonolysis reaction. A synthetic pathway towards the desired sophorolipid aldehyde intermediate **iv** was successfully developed (Scheme I). Sophorolipid lactone **i** was transformed into sophorolipid methyl ester **ii** and, subsequently, into the peracetylated analogue **iii** according to literature procedures. Cleavage of the double bond through ozonolysis resulted in sophorolipid aldehyde **iv** *via* reductive work-up. Ozonolysis of the diacetylated sophorolipid lactone **i** and the sophorolipid methyl ester **ii** was also evaluated, but proved to be not successful for the synthesis of the desired sophorolipid aldehyde intermediate.



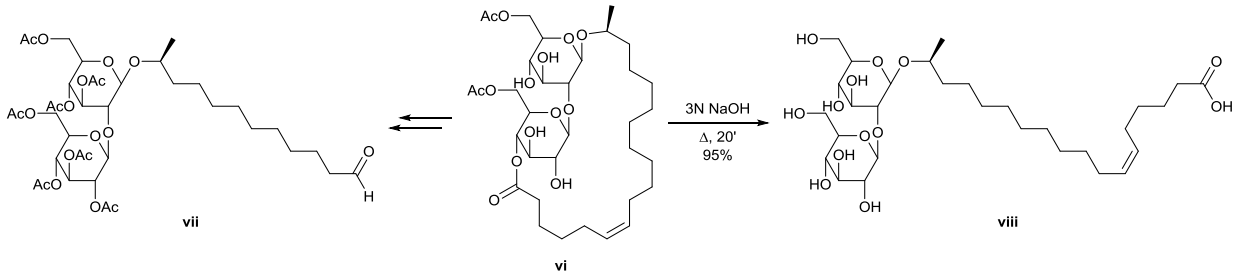
Scheme I. Optimized synthetic pathway towards sophorolipid aldehyde iv

The fermentation conditions and the concomitant purity of the starting product proved to have a big influence on the chemical derivatization. Diacetylated sophorolipid lactones **i** obtained from fermentations with the *Starmerella bombicola* oe *sble* strain, which produces selectively sophorolipid lactones, using oleic acid and yeast extract as substrates were most suitable as starting product. The synthetic pathway was extended to the production of the sophorolipid alcohol intermediate **v** by adjusting the reductive work-up of the ozonolysis reaction (Scheme II).



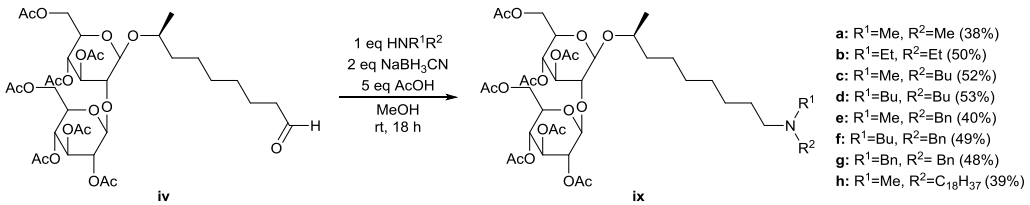
Scheme II. Synthesis of intermediate sophorolipid alcohol v

Moreover, the synthesis of the C12 sophorolipid aldehyde **vii** was accomplished *via* the incorporation of petroselinic acid in the sophorolipid structure (Scheme III). The CMC value and the corresponding surface tension of the petroselinic acid based diacetylated sophorolipid lactone **vi** and sophorolipid acid **viii** were determined and compared to their oleic acid based counterparts. Much lower CMC values were obtained for the petroselinic acid based sophorolipids compared to their oleic acid based counterparts, indicating a less compact geometry for the petroselinic acid based sophorolipids.

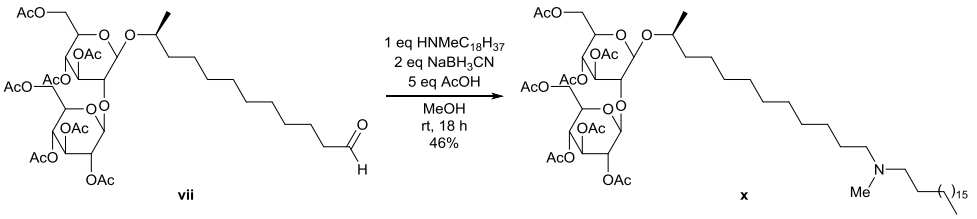


Scheme III. Synthesis of C12 sophorolipid aldehyde vii and sophorolipid acid viii

The intermediate sophorolipid aldehyde **iv** was used for the synthesis of a broad set of innovative sophorolipid derivatives. The first step in this modification pathway comprised the synthesis of sophorolipid amines **ix** *via* reductive amination with secondary amines (Scheme IV). This reductive amination was extended to sophorolipid aldehyde **vii** for the synthesis of *N*-methyl,*N*-octadecyl sophorolipid amine **x** (Scheme V).

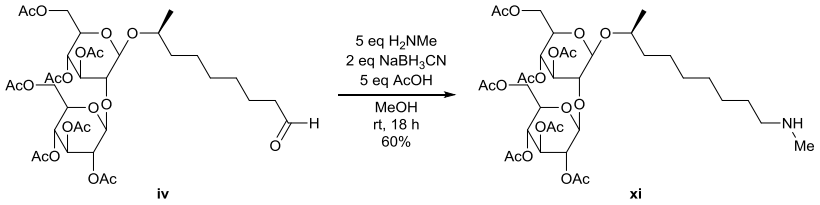


Scheme IV. Reductive amination of sophorolipid aldehyde iv towards sophorolipid amines ix



Scheme V. Reductive amination of sophorolipid aldehyde vii towards sophorolipid amine x

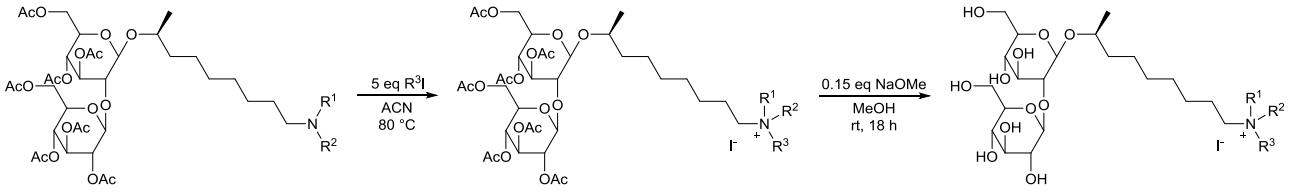
The reductive amination was extended to primary amines for the synthesis of secondary sophorolipid amines (Scheme VI). The mixture of sophorolipid aldehyde **iv** and the primary amine had to be stirred 1 hour at room temperature prior to the addition of sodium cyanoborohydride and acetic acid to avoid the dual reductive amination towards bolaamphiphilic sophorolipids.



Scheme VI. Selective synthesis of sophorolipid methyl amine xi

The purity of the sophorolipid amines after reductive amination proved to be highly dependent on the quality of the sophorolipid aldehyde. No extra purification step was needed after reductive amination with highly pure sophorolipid aldehyde **iv** which was derived from ozonolysis reactions with dichloromethane as solvent. However, in the optimized reaction procedure of the ozonolysis reaction, methanol is used as solvent instead of dichloromethane to increase the green character of the synthetic pathway. In this case, further purification proved to be necessary due to the slightly lower purity of this ozonolysis product. Therefore, the ozonolysis reaction can be addressed as the major bottleneck of the overall synthetic pathway. A balance should be found between the green character of the ozonolysis solvent and its influence on the purity and concomitant purification steps of the sophorolipid amine derivatives after reductive amination.

The sophorolipid tertiary amines **ix** were used for the synthesis of a varied set of peracetylated and deprotected quaternary ammonium sophorolipids (Scheme VII). Sophorolipid amines **ix** were quaternized with alkyl iodides in pressure vials towards the peracetylated quaternary ammonium sophorolipids **xii**. Subsequent deprotection of the sugar head groups with sodium methoxide yielded the deprotected quaternary ammonium sophorolipids **xiii**. This quaternization and deprotection procedure was extended to *N*-methyl,*N*-octadecyl sophorolipid amine **x** for the synthesis of peracetylated and deprotected quaternary ammonium sophorolipids **xiv** and **xv** (Scheme VIII).



ix

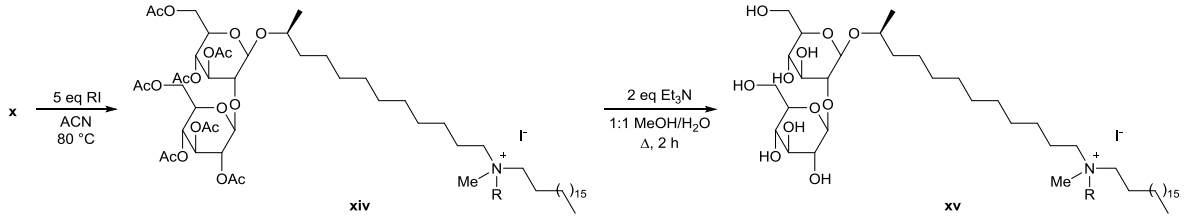
xii

a: R^1=Me, R^2=Me, R^3=Me (91%)
b: R^1=Me, R^2=Me, R^3=Bu (89%)
c: R^1=Me, R^2=Bu, R^3=Bu (96%)
d: R^1=Bu, R^2=Bu, R^3=Bu (94%)
e: R^1=Me, R^2=Me, R^3=Bn (quant.)
f: R^1=Me, R^2=Bu, R^3=Bn (89%)
g: R^1=Me, R^2=Bn, R^3=Bn (quant.)
h: R^1=Me, R^2=Me, R^3=$C_{18}H_{37}$ (98%)
i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$ (quant.)

xiii

a: R^1=Me, R^2=Me, R^3=Me (quant.)
b: R^1=Me, R^2=Me, R^3=Bu (88%)
c: R^1=Me, R^2=Bu, R^3=Bu (quant.)
d: R^1=Bu, R^2=Bu, R^3=Bu (quant.)
e: R^1=Me, R^2=Me, R^3=Bn (quant.)
f: R^1=Me, R^2=Bu, R^3=Bn (quant.)
g: R^1=Me, R^2=Bn, R^3=Bn (99%)
h: R^1=Me, R^2=Me, R^3=$C_{18}H_{37}$ (97%)
i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$ (66%)

Scheme VII. Synthesis of peracetylated and deprotected sophorolipid amines xii and xiii



xiv

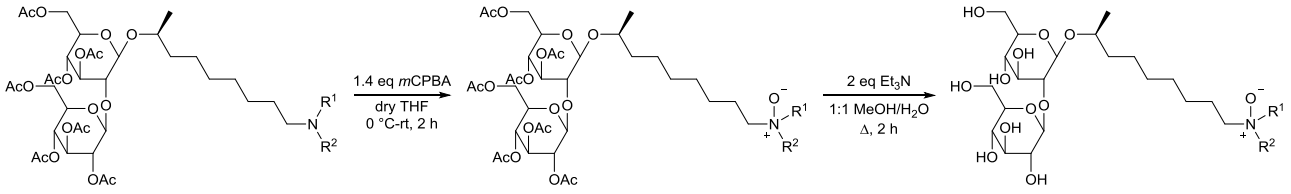
a: R=Me (99%)
b: R=Bu (97%)

xv

a: R=Me (70%)
b: R=Bu (19%)

Scheme VIII. Synthesis of peracetylated and deprotected sophorolipid amines xiv and xv

The sophorolipid tertiary amines **ix** were also used for the synthesis of a varied set of peracetylated and deprotected sophorolipid amine oxides (Scheme IX). Sophorolipid amines **ix** were oxidized with *m*CPBA towards the peracetylated sophorolipid amine oxides **xvi**. Subsequent deprotection of the sugar head groups with triethylamine in a water/methanol mixture yielded the deprotected sophorolipid amine oxides **xvii**.



ix

xvi

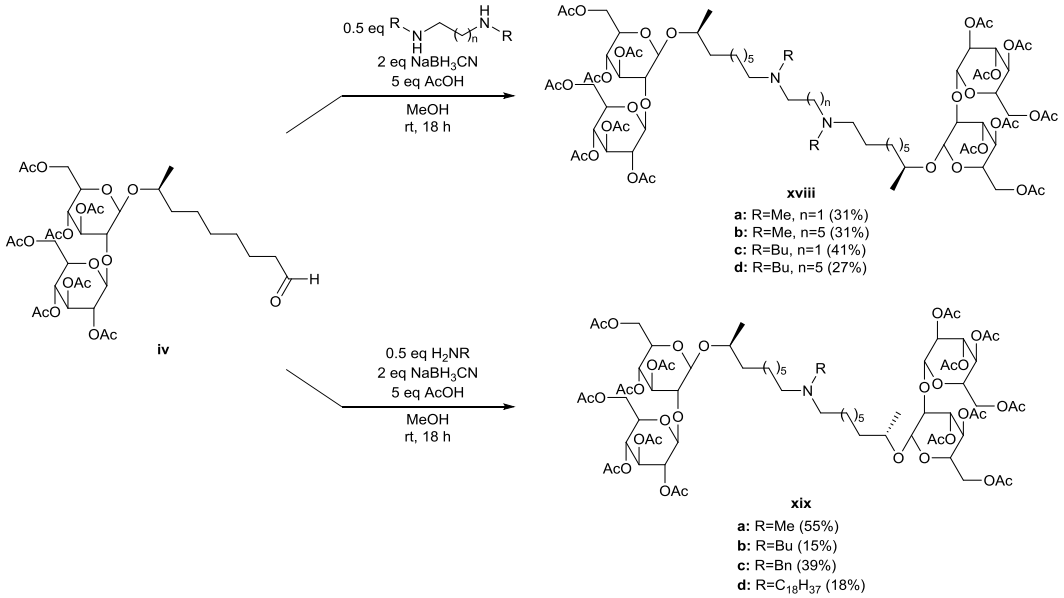
a: R^1=Me, R^2=Me (79%)
b: R^1=Me, R^2=Bu (87%)
c: R^1=Bu, R^2=Bu (93%)
d: R^1=Me, R^2=Bn (95%)
e: R^1=Bu, R^2=Bn (94%)
f: R^1=Bn, R^2=Bn (63%)
g: R^1=Me, R^2=$C_{18}H_{37}$ (92%)

xvii

a: R^1=Me, R^2=Me (quant.)
b: R^1=Me, R^2=Bu (95%)
c: R^1=Bu, R^2=Bu (84%)
d: R^1=Me, R^2=Bn (quant.)
e: R^1=Bu, R^2=Bn (91%)
f: R^1=Bn, R^2=Bn (quant.)
g: R^1=Me, R^2=$C_{18}H_{37}$ (81%)

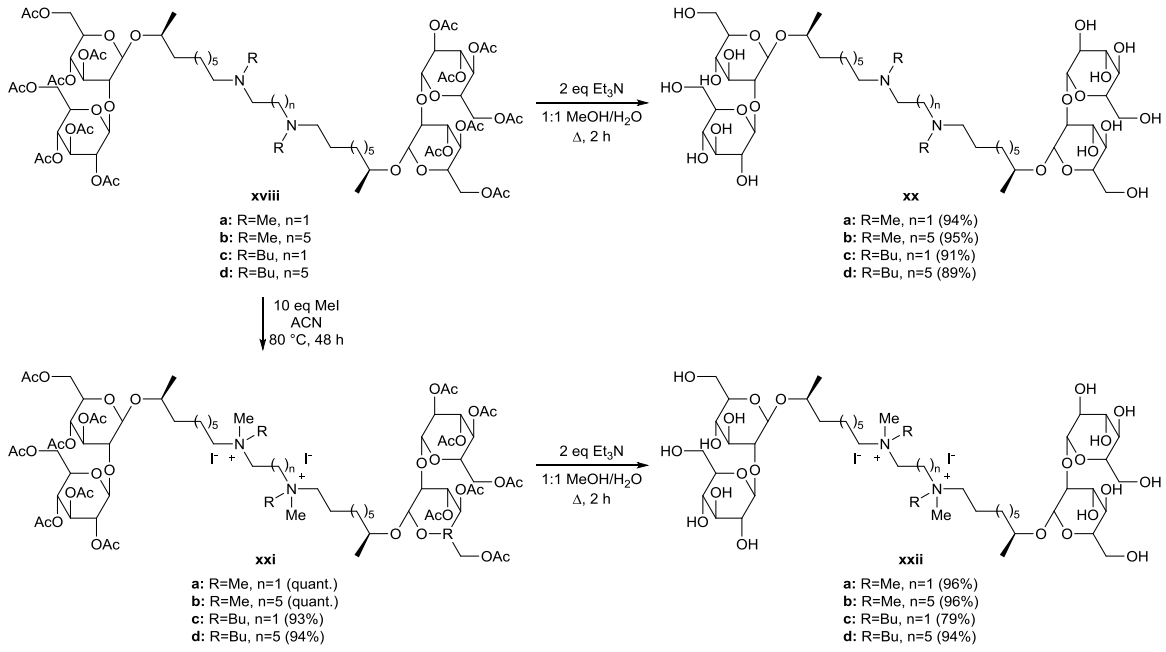
Scheme IX. Synthesis of peracetylated and deprotected sophorolipid amine oxides xvi and xvii

Two different sets of bolaamphiphilic sophorolipid amines were synthesized from sophorolipid aldehyde **iv** (Scheme X). Peracetylated *N*,*N*'-dialkyl bolaamphiphilic sophorolipids **xviii** were synthesized *via* reductive amination with different *N*,*N*'-dialkyl diamines. Several attempts to synthesize *N*,*N*'-dioctadecyl bolaamphiphilic sophorolipids were unsuccessful. Peracetylated *N*-alkyl bolaamphiphilic sophorolipids **xix** were synthesized *via* reductive amination with different primary amines.

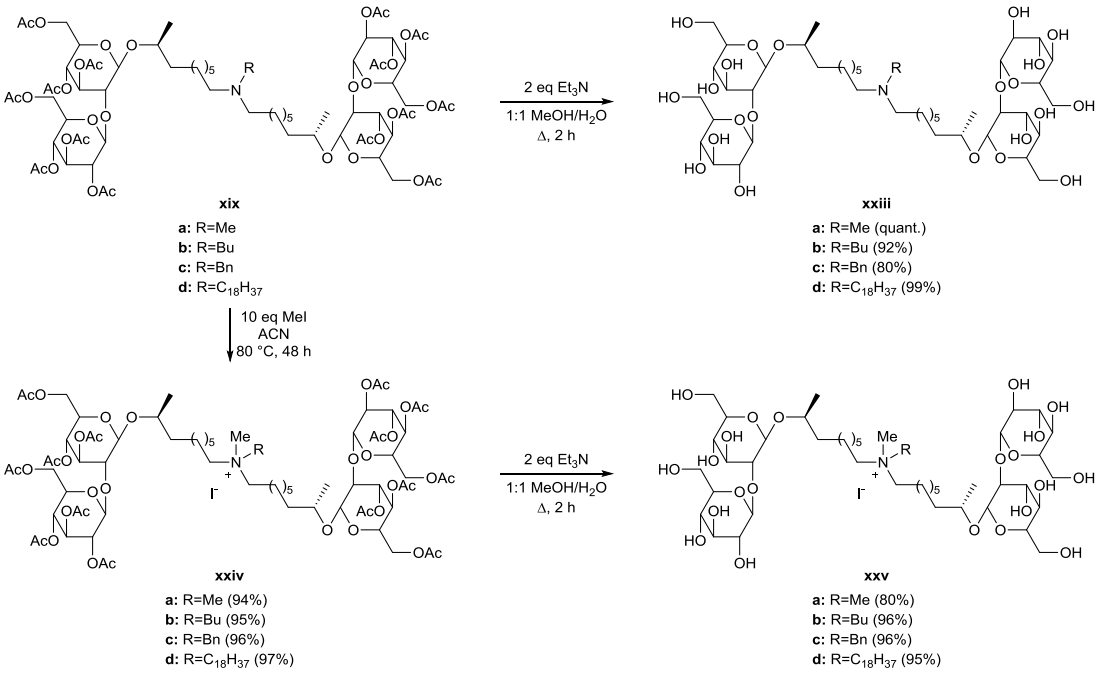


Scheme X. Synthesis of peracetylated *N,N'*-dialkyl and *N*-alkyl bolaamphiphilic sophorolipids xviii and xix

The peracetylated bolaamphiphilic sophorolipid amines **xviii** and **xix** were transformed into a broad set of bolaamphiphilic sophorolipid derivatives (Scheme XI, Scheme XII). Deprotection with triethylamine in a water/methanol mixture yielded deprotected bolaamphiphilic sophorolipid amines **xx** and **xxiii**. Quaternization with methyl iodide in a pressure vial resulted in the synthesis of cationic bolaamphiphilic sophorolipids **xxi** and **xxiv**. Finally, these cationic bolaamphiphilic sophorolipids were deprotected with triethylamine in a water/methanol mixture into deprotected cationic bolaamphiphilic sophorolipids **xxii** and **xxv**.



Scheme XI. Modification of *N,N'*-dialkyl bolaamphiphilic sophorolipids xviii *via* quaternization and deprotection



Scheme XII. Modification of *N*-alkyl bolaamphiphilic sophorolipids xix *via* quaternization and deprotection

All derivatives have been evaluated for their antimicrobial activity against a set of Gram-positive and Gram-negative strains (Table I). None of the derivatives displayed significant activity against any of the Gram-negative strains. The quaternary ammonium sophorolipids, monocationic bolaamphiphilic sophorolipids and dicationic bolaamphiphilic sophorolipids proved to be the most active derivatives. The best results were obtained for the deprotected quaternary ammonium sophorolipids **xiii(h)** and **xiii(i)**. These derivatives were even more active than the antibiotic gentamicin sulfate against the four Gram-positive strains *S. aureus*, *E. faecium*, *B. subtilis* and *S. pneumoniae* in *in vitro* evaluations. Moreover, evaluation of the deglycosylated derivatives of these two quaternary ammonium sophorolipids demonstrated that the presence of the carbohydrate head has a positive effect on the antimicrobial activity.

Evaluation of the quaternary ammonium sophorolipids on their suitability as gene delivery vectors indicated that the same two quaternary ammonium sophorolipids **xiii(h)** and **xiii(i)** were the most effective derivatives. Moreover, evaluation of their deglycosylated derivatives demonstrated that the presence of the carbohydrate head highly increased the cell viability of the evaluated cell lines and concomitant biocompatibility of these quaternary ammonium sophorolipids. Small-angle X-ray scattering (SAXS) analysis demonstrated that only the two quaternary ammonium sophorolipids **xiii(h)** and **xiii(i)** formed spherical micelles. These specific self-assembly properties and increased transfection efficiency may be attributed to the presence of the long aliphatic chain in the quaternary ammonium sophorolipids.

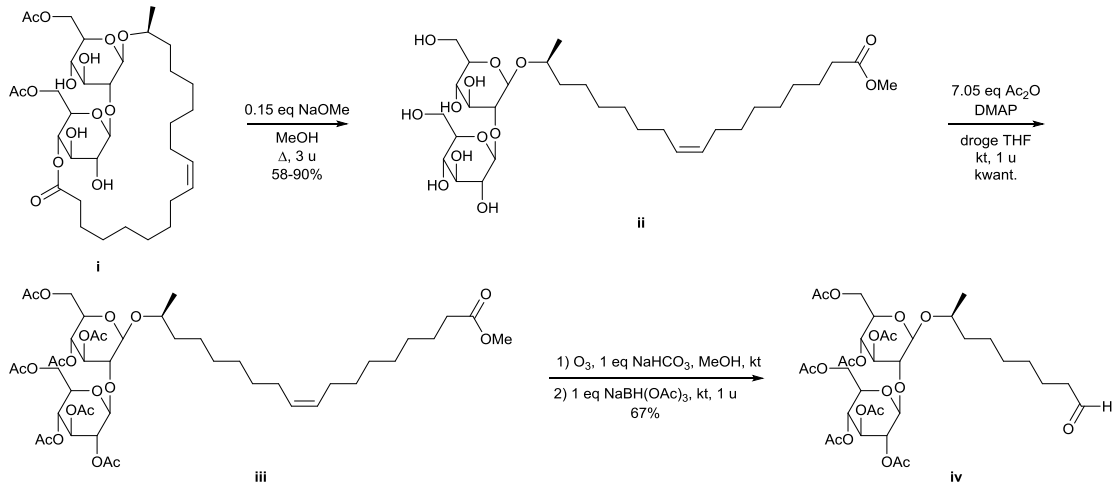
Table I. Overview of the minimum inhibitory concentrations (µM) of the different sophorolipid derivatives

	S. aureus	*S. aureus* Mu50	*E. faecium*	*B. subtilis*	*S. pneumoniae*
i	45.4	90.79			
vi	45.4	363.2			
viii	>1607	>1607			
xii(b)	>101		>101	>101	>101
xii(c)	>97		>97	24	97
xii(d)	>93		>93	>93	>93
xii(e)	489		>977	977	977
xii(f)	>94		>94	>94	>94
xii(g)	45		>91	45	91
xii(h)	6.59-8	26.36	8	8	8
xii(i)	6.36-8	50.91	8	8	8
xiii(b)	>144		>144	>144	>144
xiii(h)	2.18-6	4.37	6	6	6
xiii(i)	2.09-5	4.18	5	5	5
xiv(a)	12.73	815			
xiv(b)	12.31	394			
xv(a)	66.9	66.9			
xv(b)	23.03	64.1			
xviii(b)	375	1501			
xxi(a)	165	660			
xxi(b)	80	321			
xxi(c)	20	158			
xxi(d)	38	38			
xxii(b)	392	>1569			
xxiv(a)	92	184			
xxiv(b)	45	45			
xxiv(c)	44	44			
xxv(c)	132	>2114			
xxv(d)	116	232			

6. Samenvatting

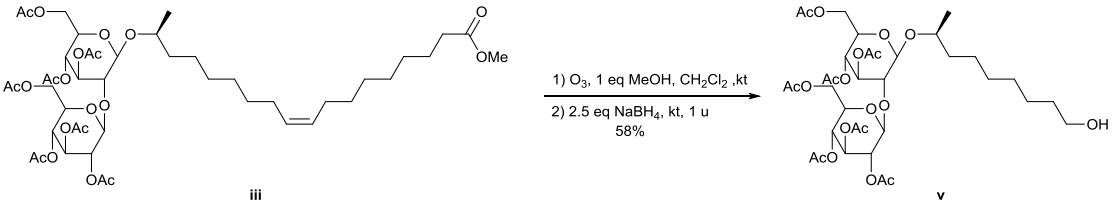
Sophorolipiden, een klasse van glycolipide biosurfactants, zijn uitermate interessante hernieuwbare bouwstenen voor chemische derivatisering. Ze worden fermentatief geproduceerd door micro-organismen waarbij de gist *Starmerella bombicola* bij voorkeur gebruikt wordt als productie organisme. Sophorolipiden voldoen aan alle criteria om beschouwd te worden als hernieuwbare bouwstenen voor chemische derivatisering aangezien ze beschikken over een complexe structuur in combinatie met hoge productiehoeveelheden en zuiverheid. Een opbrengst rond 400 g/L kan worden verkregen aan een geschatte productieprijs van 2 tot 5 €/kg. Verschillende sophorolipide derivaten kunnen geproduceerd worden *via* fermentatie en met genetisch gemodificeerde *S. bombicola* stammen kan de selectieve productie van één sophorolipide derivaat verkregen worden. Natuurlijke sophorolipiden bezitten zelf reeds interessante biologische en fysico-chemische eigenschappen. Ze beschikken over anti-kanker, antimicrobiële, dermatologische, immunoregulatorische, spermicidale en antivirale eigenschappen. Ze bezitten ook *self-assembly* eigenschappen met een grote variatie in de aard van de nanostructuren die gevormd worden door de verschillende sophorolipiden.

In dit werk werd het belangrijkste fermentatieproduct, namelijk het digeacetyleerde sophorolipide lacton, gebruikt voor de synthese van een uitgebreide reeks aan innovatieve sophorolipide derivaten. Het eerste doel omsloot de synthese van korte keten intermediaire sophorolipide derivaten, meer bepaald een sophorolipide aldehyde, *via* een ozonolyse reactie. Met succes werd een synthetische pathway voor het gewenste sophorolipide aldehyde intermediair **iv** ontwikkeld (Schema I). Het sophorolipide lacton **i** werd omgezet naar het sophorolipide methyl ester **ii** en vervolgens naar het pergeacetyleerde analoog **iii** volgens bestaande procedures. Het sophorolipide aldehyde **iv** werd verkregen door splitsing van de dubbele binding *via* ozonolyse gevolgd door reductieve opwerking. Ozonolyse van het digeacetyleerd sophorolipide lacton **i** en het sophorolipide methyl ester **ii** werd ook geëvalueerd, maar leidde niet tot de synthese van het gewenste aldehyde intermediair.



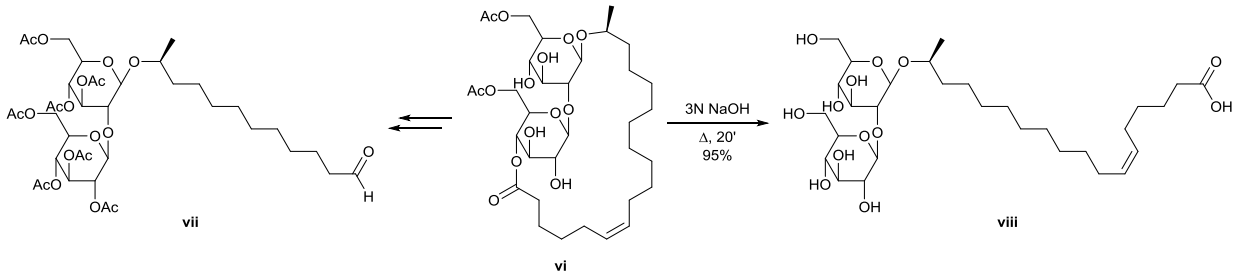
Schema I. Geoptimaliseerde synthetische pathway naar sophorolipide aldehyde iv

De fermentatie condities en de daaruit volgende zuiverheid van het startproduct hebben een grote invloed op de chemische derivatisering. Digeacetyleerd sophorolipide lacton **i** verkregen uit fermentaties met de *S. bombicola* oe *sble* stam, die selectief sophorolipide lactonen produceert, zijn het meest geschikt als startproduct wanneer oleïnezuur en gistextract gebruikt worden als substraat. De synthetische pathway werd uitgebreid naar de productie van het sophorolipide alcohol intermediair **v** door de reductieve opwerking van de ozonolyse reactie aan te passen (Schema II).



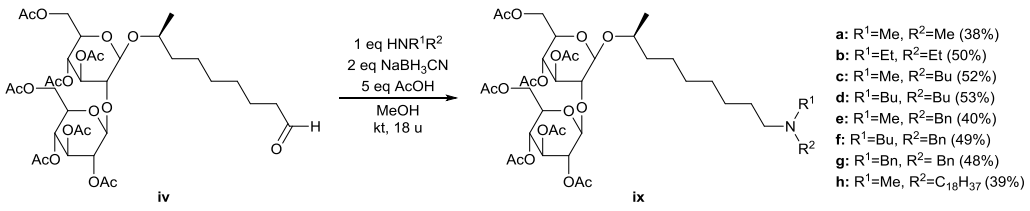
Schema II. Synthese van het intermediaire sophorolipide alcohol v

Daarnaast werd ook de synthese van een C12 sophorolipide aldehyde **vii** verkregen door het inbouwen van petroselinezuur in de sophorolipide structuur (Schema III). De CMC waarde en bijhorende oppervlaktespanning van de petroselinezuur gebaseerde digeacetyleerde sophorolipide lacton **vi** en het zure sophorolipide **viii** werden bepaald en vergeleken met de overeenkomstige oleïnezuur derivaten. De petroselinezuur gebaseerde sophorolipiden hebben een veel lagere CMC waarde dan hun oleïnezuur gebaseerde tegenhangers, wat wijst op een minder compacte geometrie voor de petroselinezuur gebaseerde sophorolipiden.

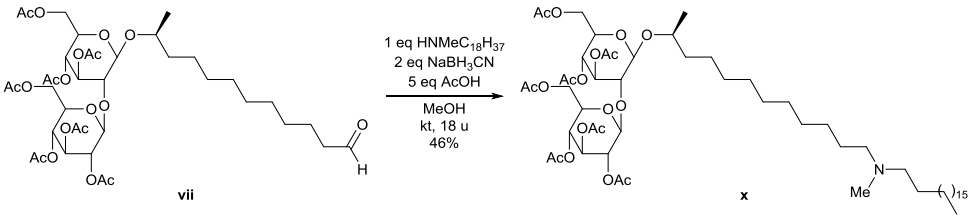


Schema III. Synthese van een C12 sophorolipide aldehyde vii en zuur sophorolipide viii

Het intermediaire sophorolipide aldehyde **iv** werd gebruikt voor de synthese van een brede waaier aan innovatieve sophorolipide derivaten. Als eerste stap in de modificatie pathway werden sophorolipide amines **ix** gevormd *via* reductieve aminering met secundaire amines (Schema IV). Deze reductieve aminering werd ook toegepast op sophorolipide **vii** voor de synthese van *N*-methyl,*N*-octadecyl sophorolipide amine **x** (Schema V).

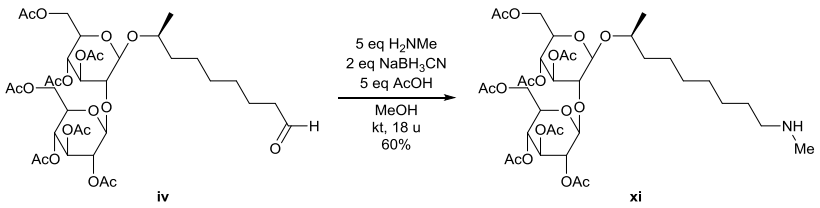


Schema IV. Reductieve aminering van sophorolipide aldehyde iv naar sophorolipide amines ix



Schema V. Reductieve aminering van sophorolipide aldehyde vii naar sophorolipide amine x

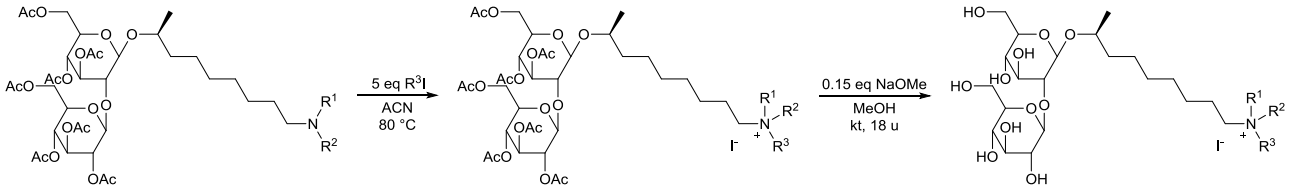
Reductieve aminering werd ook toegepast voor de synthese van secundaire sophorolipide amines aan de hand van primaire amines (Schema VI). Het roeren van sophorolipide aldehyde **iv** en het primaire amine gedurende 1 uur bij kamertemperatuur voor het toevoegen van natrium cyanoboorhydride en azijnzuur bleek noodzakelijk om een extra reductieve aminering tot bolaamfifiele sophorolipiden te voorkomen.



Schema VI. Selectieve synthese van sophorolipide methyl amine xi

De zuiverheid van de sophorolipide amines na de reductieve aminering was sterk afhankelijk van de kwaliteit van het sophorolipide aldehyde. Na de reductieve aminering met zeer zuiver sophorolipide aldehyde **iv**, verkregen uit ozonolyse reactie met dichloormethaan als solvent, was er geen extra opzuiveringsstap nodig. In de geoptimaliseerde reactieprocedure van de ozonolyse reactie wordt er echter methanol gebruikt in de plaats van dichloormethaan om het groene karakter van de synthetische pathway te verhogen. In dit geval was verdere opzuivering noodzakelijk door de verminderde zuiverheid van het ozonolyse product. Hierdoor kan de ozonolyse reactie aangeduid worden als knelpunt van de synthetische pathway. Er moet een balans worden gevonden tussen het groene karakter van het solvent voor de ozonolyse en zijn invloed op de zuiverheid en de daaruitvolgende opzuiveringsstappen van de sophorolipide amines na de reductieve aminering.

De tertiaire sophorolipide amines **ix** werden gebruikt voor de synthese van een gevarieerde set aan pergeacetyleerde en ontschermde sophorolipide quaternaire ammoniumzouten (Schema VII). De sophorolipide amines **ix** werden gequaterniseerd met alkyl iodides in drukvaten tot de pergeacetyleerde sophorolipide quaternaire ammoniumzouten **xii**. De daaropvolgende ontscherming van de suikerkop werd uitgevoerd met natrium methoxide tot de ontschermde sophorolipide quaternaire ammoniumzouten **xiii**. Deze quaternisering en ontscherming werden uitgebreid naar het *N*-methyl,*N*-octadecyl sophorolipide amine **x** voor de synthese van de pergeacetyleerde en ontschermde sophorolipide quaternaire ammoniumzouten **xiv** en **xv** (Schema VIII).



ix

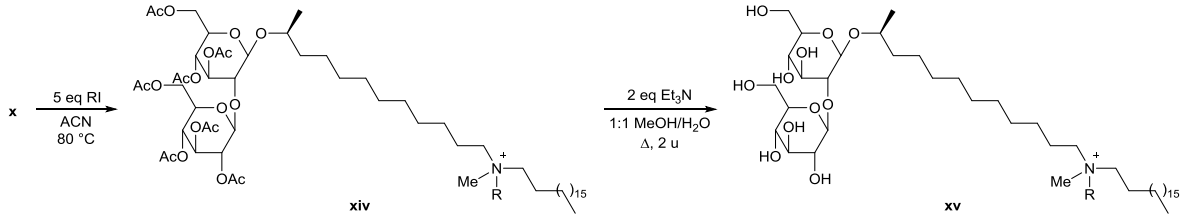
xii

a: R^1=Me, R^2=Me, R^3=Me (91%)
b: R^1=Me, R^2=Me, R^3=Bu (89%)
c: R^1=Me, R^2=Bu, R^3=Bu (96%)
d: R^1=Bu, R^2=Bu, R^3=Bu (94%)
e: R^1=Me, R^2=Me, R^3=Bn (quant.)
f: R^1=Me, R^2=Bu, R^3=Bn (89%)
g: R^1=Me, R^2=Bn, R^3=Bn (quant.)
h: R^1=Me, R^2=Me, R^3=$C_{18}H_{37}$ (98%)
i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$ (quant.)

xiii

a: R^1=Me, R^2=Me, R^3=Me (quant.)
b: R^1=Me, R^2=Me, R^3=Bu (88%)
c: R^1=Me, R^2=Bu, R^3=Bu (quant.)
d: R^1=Bu, R^2=Bu, R^3=Bu (quant.)
e: R^1=Me, R^2=Me, R^3=Bn (quant.)
f: R^1=Me, R^2=Bu, R^3=Bn (quant.)
g: R^1=Me, R^2=Bn, R^3=Bn (99%)
h: R^1=Me, R^2=Me, R^3=$C_{18}H_{37}$ (97%)
i: R^1=Me, R^2=Bu, R^3=$C_{18}H_{37}$ (66%)

Schema VII. Synthese van pergeacetyleerde en ontschermde sophrolipide amines xii en xiii



xiv

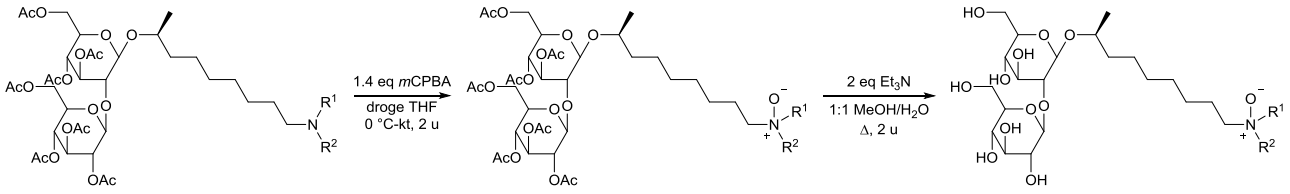
a: R=Me (99%)
b: R=Bu (97%)

xv

a: R=Me (70%)
b: R=Bu (19%)

Schema VIII. Synthese van pergeacetyleerde en ontschermde sophorolipide amines xiv en xv

De tertiaire sophorolipide amines **ix** werden ook gebruikt voor de synthese van een uitgebreide set aan pergeacetyleerde en ontschermde sophorolipide amine oxides (Schema IX). De sophorolipide amines **ix** werden geoxideerd met *m*CPBA tot de pergeacetyleerde sophorolipide amine oxides **xvi**. De daaropvolgende ontscherming van de suikergroepen werd uitgevoerd met triethylamine in een water/methanol mengsel tot de ontschermde sophorolipide amine oxides **xvii**.



ix

xvi

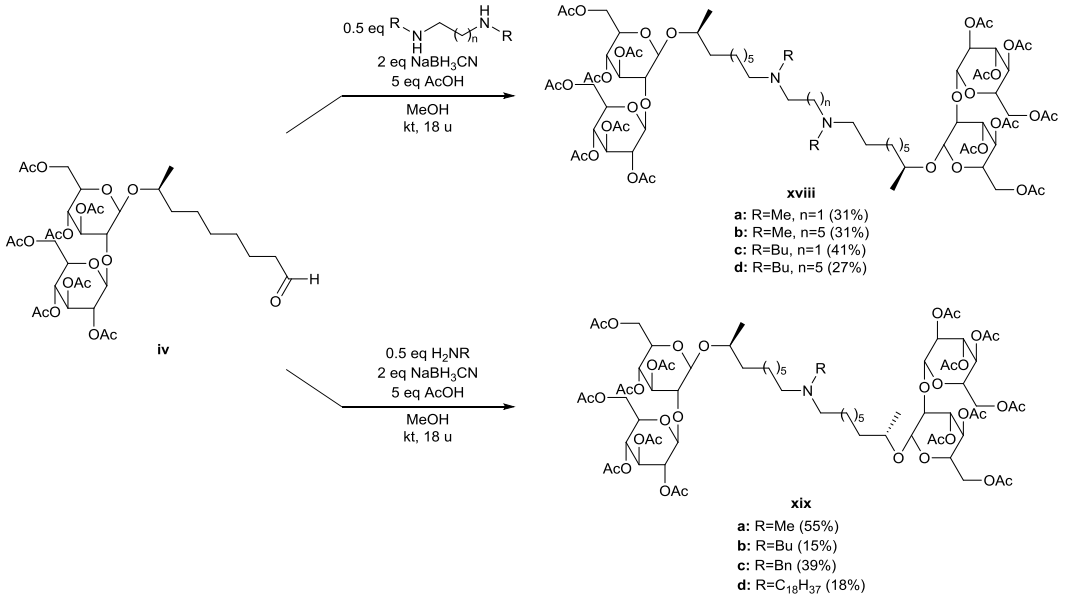
a: R^1=Me, R^2=Me (79%)
b: R^1=Me, R^2=Bu (87%)
c: R^1=Bu, R^2=Bu (93%)
d: R^1=Me, R^2=Bn (95%)
e: R^1=Bu, R^2=Bn (94%)
f: R^1=Bn, R^2=Bn (63%)
g: R^1=Me, R^2=$C_{18}H_{37}$ (92%)

xvii

a: R^1=Me, R^2=Me (quant.)
b: R^1=Me, R^2=Bu (95%)
c: R^1=Bu, R^2=Bu (84%)
d: R^1=Me, R^2=Bn (quant.)
e: R^1=Bu, R^2=Bn (91%)
f: R^1=Bn, R^2=Bn (quant.)
g: R^1=Me, R^2=$C_{18}H_{37}$ (81%)

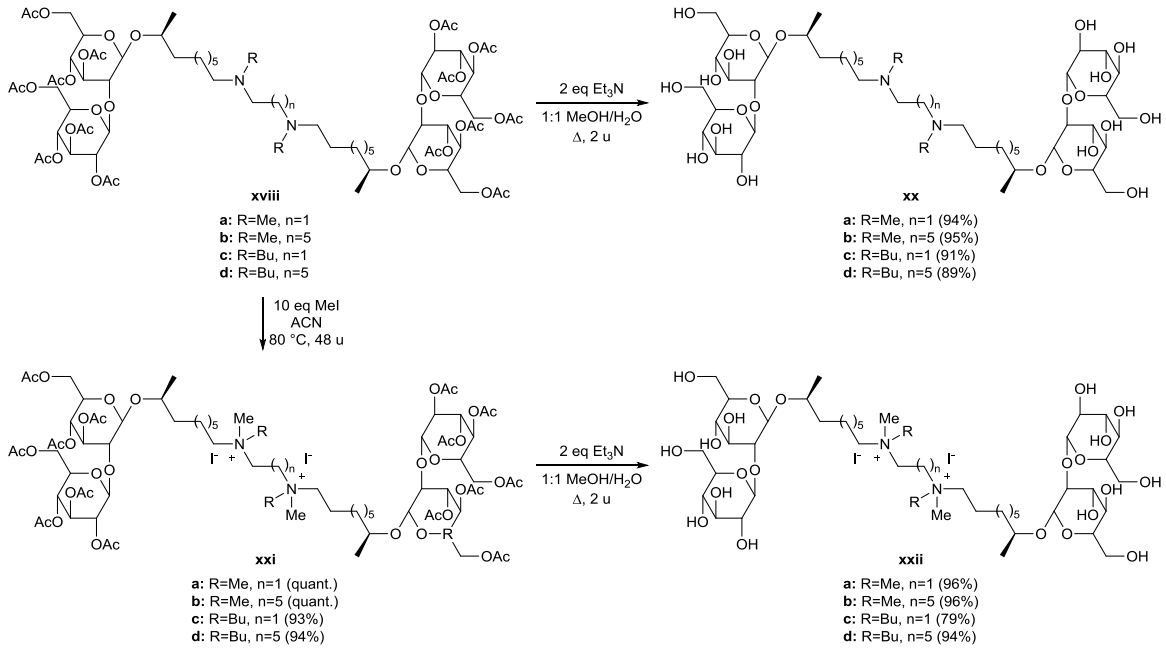
Schema IX. Synthese van pergeacetyleerde en ontschermde sophorolipide amine oxides xvi en xvii

Twee verschillende klassen aan bolaamfifiele sophorolipide amines werden gevormd uit sophorolipide aldehyde **iv** (Schema X). Pergeacetyleerde *N*,*N*'-dialkyl bolaamfifiele sophorolipiden **xviii** werden verkregen *via* reductieve aminering met verschillende *N*,*N*'-dialkyl diamines. Verschillende pogingen voor de synthese van *N*,*N*'-dioctadecyl bolaamfifiele sophorolipiden waren niet succesvol. Pergeacetyleerde *N*-alkyl bolaamfifiele sophorolipiden **xix** werden gevormd *via* reductieve aminering met verschillende primaire amines.

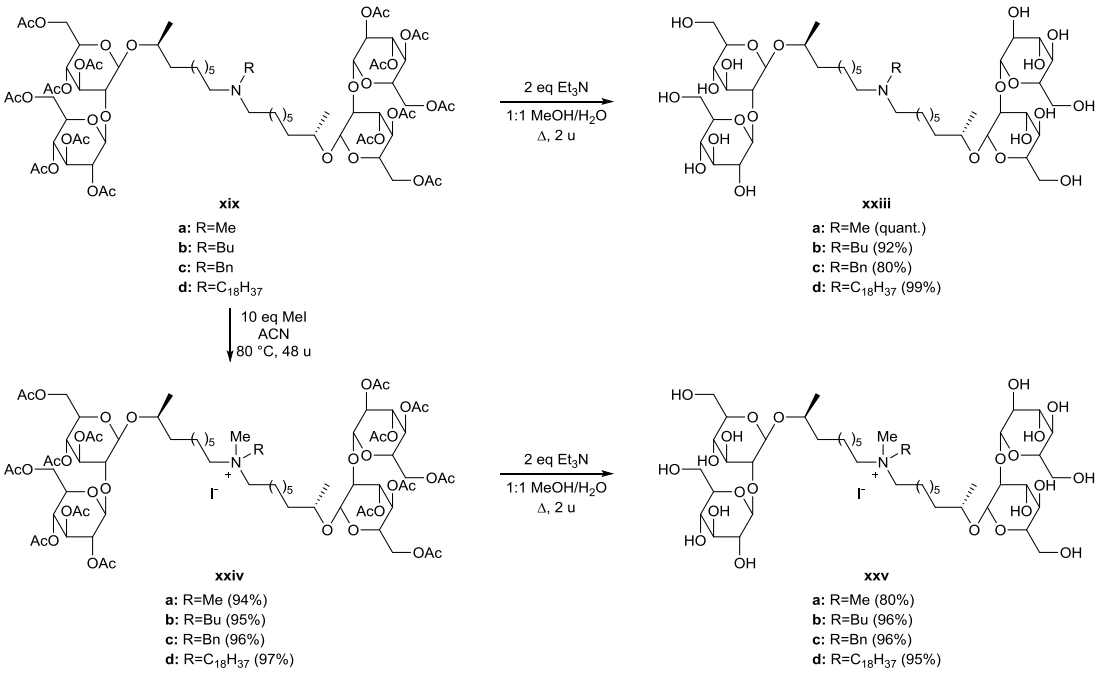


Schema X. Synthese van pergeacetyleerde *N,N'*-dialkyl en *N*-alkyl bolaamfifiele sophorolipiden xviii en xix

De pergeacetyleerde bolaamfifiele sophorolipide amines **xviii** en **xix** werden omgezet in een uitgebreide groep aan bolaamfifiele sophorolipide derivaten (Schema XI, Schema XII). De ontscherming werd uitgevoerd met triëthylamine in een water/methanol mengsel tot de ontschermde bolaamfifiele sophorolipide amines **xx** en **xxiii**. De quaternisering werd uitgevoerd met methyl iodide in een drukvat tot de kationische bolaamfifiele sophorolipiden **xxi** en **xxiv**. Tot slot werden de kationische bolaamfifiele sophorolipiden ontschermd met triëthylamine in een water/methanol mengsel tot de ontschermde kationische bolaamfifiele sophorolipiden **xxii** en **xxv**.



Schema XI. Modificatie van *N,N'*-dialkyl bolaamfifiele sophorolipiden xviii *via* quaternisering en ontscherming



Schema XII. Modificatie van *N*,*N*'-dialkyl bolaamfifiele sophorolipiden xix *via* quaternisering en ontscherming

Alle derivaten werden geëvalueerd op hun antimicrobiële activiteit tegen een groep Gram-positieve en Gram-negatieve stammen (Tabel I). Geen enkele van de derivaten vertoonde significante activiteit tegen één van de Gram-negatieve stammen. De sophorolipide quaternaire ammoniumzouten, monokationische bolaamfifiele sophorolipiden en dikationische bolaamfifiele sophorolipiden zijn de meest actieve derivaten. De beste resultaten werden verkregen voor de ontschermde sophorolipide quaternaire ammoniumzouten **xiii(h)** en **xiii(i)**. Deze derivaten waren zelfs actiever dan het antibioticum gentamicine sulfaat tegen de vier Gram-positieve stammen *S. aureus*, *E. faecium*, *B. subtilis* en *S. pneumoniae* in een *in vitro* evaluatie. Daarnaast toonde de evaluatie van de gedeglycosyleerde derivaten van deze sophorolipide quaternaire ammoniumzouten aan dat de aanwezigheid van de suikerkop een positief effect heeft op de antimicrobiële activiteit.

De evaluatie van de quaternaire ammoniumzouten als *gene delivery* vectoren toonde aan dat dezelfde twee sophorolipide quaternaire ammoniumzouten **xiii(h)** en **xiii(i)** de hoogste activiteit vertoonden. Daarnaast toonde de evaluatie van hun gedeglycosyleerde derivaten aan dat de aanwezigheid van de suikerkop de levensvatbaarheid van de cellen en de daaruitvolgende bio-compatibiliteit met de geëvalueerde cellijnen sterk verhoogde. *Small-angle X-ray scattering* (SAXS) analyse toonde aan dat enkel de sophorolipide quaternaire ammoniumzouten **xiii(h)** en **xiii(i)** sferische micellen vormen. Deze specifieke *self-assembly* eigenschappen en verhoogde transfectie-efficiëntie kunnen toegewezen worden aan de aanwezigheid van de lange alifatische keten in deze sophorolipide quaternaire ammoniumzouten.

Tabel I. Overzicht van de minimale inhibitie concentraties (μM) voor de verschillende sophorolipide derivaten

	S. aureus	*S. aureus* Mu50	*E. faecium*	*B. subtilis*	*S. pneumoniae*
i	45.4	90.79			
vi	45.4	363.2			
viii	>1607	>1607			
xii(b)	>101		>101	>101	>101
xii(c)	>97		>97	24	97
xii(d)	>93		>93	>93	>93
xii(e)	489		>977	977	977
xii(f)	>94		>94	>94	>94
xii(g)	45		>91	45	91
xii(h)	6.59-8	26.36	8	8	8
xii(i)	6.36-8	50.91	8	8	8
xiii(b)	>144		>144	>144	>144
xiii(h)	2.18-6	4.37	6	6	6
xiii(i)	2.09-5	4.18	5	5	5
xiv(a)	12.73	815			
xiv(b)	12.31	394			
xv(a)	66.9	66.9			
xv(b)	23.03	64.1			
xviii(b)	375	1501			
xxi(a)	165	660			
xxi(b)	80	321			
xxi(c)	20	158			
xxi(d)	38	38			
xxii(b)	392	>1569			
xxiv(a)	92	184			
xxiv(b)	45	45			
xxiv(c)	44	44			
xxv(c)	132	>2114			
xxv(d)	116	232			

7. Curriculum vitae

Personalia

Name	Elisabeth Delbeke
Adress	Folke Bernadottestraat 8A – 9000 Ghent
Email	Elisabeth.Delbeke@UGent.be; delbekeelisabeth@gmail.com
Mobile phone	+32 (0)497 72 15 32
Date of Birth	19/09/1989
Place of Birth	Ghent, Belgium
Nationality	Belgian
Civil State	Legally cohabiting

Education

2010-2012 ***Master of Science in Bioscience Engineering***
Chemistry and Bioprocess Technology, Faculty of Bioscience Engineering, Ghent University, Greatest distinction

Master thesis: "*Synthesis of biologically relevant diketopiperazine scaffolds*"
Department of Sustainable Organic Chemistry and Technology
Academic year 2011-2012
Promotor: Prof. dr. ir. Chris Stevens

Internship: "*HACCP-analysis for chocolate, nuts and coffee from Oxfam Fairtrade*"
Oxfam Fairtrade cvba
Academic year 2010-2011

2007-2010 ***Bachelor of Science in Bioscience Engineering***
Chemistry and Food Technology, Faculty of Bioscience Engineering, Ghent University, Distinction

Bachelor thesis: "*Development and validation of QSARs for mixtures of chemical compounds"*
Department of Applied Ecology and Environmental Biology
Department of Applied Mathematics, Biometrics and Process Control
Academic year 2009-2010
Promotors: Prof. dr. Colin Janssens & Prof. dr. ir. Olivier Thas

2001-2007 ***General Secondary Education***
Latin-Mathematics (8h), Humaniora Nieuwen-Bosch, Ghent, graduated with great distinction

Employment

2012-present PhD researcher at the SynBioC research group, Faculty of Bioscience Engineering, Ghent University
Topic: *Synthesis of innovative sophorolipid analogues with tailor-made physico-chemical properties*
Promotors: Prof. dr. ir. Chris Stevens & Prof. dr. ir. Kevin Van Geem

Articles

- N. Baccile, M. Selmane, P. Le Griel, S. Prévost, J. Perez, C.V. Stevens, E. Delbeke, S. Zibek, M. Guenther, W. Soetaert, I.N.A. Van Bogaert, S. Roelants, *Langmuir*, accepted. pH-driven self-assembly of acidic microbial glycolipids.
- E. Uitterhaegen, K.A. Sampaio, E.I.P. Delbeke, W. De Greyt, M. Cerny, P. Evon, O. Merah, T. Talou, C.V. Stevens, *J. Food Process. Preserv.*, submitted. Characterization of coriander vegetable oil from French origin.
- E.I.P. Delbeke, S.L.K.W. Roelants, N. Matthijs, B. Everaert, W. Soetaert, T. Coenye, K.M. Van Geem, C.V. Stevens, *Ind. Eng. Chem. Res.*, accepted. Sophorolipid amine oxide production by a combination of fermentation scale-up and chemical modification.
- M. Movsisyan, E.I.P. Delbeke, J. Berton, C.V. Stevens, *Chem. Soc. Rev.*, synopsis accepted. Safe handling of hazardous chemistry by continuous flow technology.
- E.I.P. Delbeke, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.-A. Jaffrès, K.M. Van Geem, C.V. Stevens, *Org. Biomol. Chem.*, 2016, **14**, 3744-3751. Evaluation of the transfection efficacies of quaternary ammonium salts prepared from sophorolipids.
- E.I.P. Delbeke, J. Everaert, E. Uitterhaegen, S. Verweire, A. Verlee, T. Talou, W. Soetaert, I.N.A. Van Bogaert, C.V. Stevens, *AMB express*, 2016, **6**:28, DOI: 10.1186/s13586-016-0199-7. Petroselinic acid purification and its use for the fermentation of new sophorolipids.
- E.I.P. Delbeke, M. Movsisyan, K.M. Van Geem, C.V. Stevens, *Green Chem.*, 2016, **18**, 76-104. Chemical and enzymatic modification of sophorolipids.
- I. Wauters, H. Goossens, E. Delbeke, K. Muylaert, B.I. Roman, K. Van Hecke, V. Van Speybroeck, C.V. Stevens, *J. Org. Chem*., 2015, **80**, 8046-8054. Beyond the diketopiperazine family with alternatively bridged brevianamide F analogues.
- E.I.P. Delbeke, B.I. Roman, G.B. Marin, K.M. Van Geem, C.V. Stevens, *Green Chem.*, 2015, **17**, 3373-3377. A new class of antimicrobial biosurfactants: quaternary ammonium sophorolipids.

Participation in Conferences

- 1st International Electronic Conference on Medicinal Chemistry, 2-27/11/2015
  Keynote presentation: E.I.P. Delbeke, B.I. Roman, S.L.K.W. Roelants, I.N.A. Van Bogaert, G.B. Marin, K.M. Van Geem, C.V. Stevens, *Quaternary ammonium sophorolipids as renewable based antimicrobial products*
- 106th AOCS Annual Meeting and Industry Showcases, 3-6/05/2015, Orlando, USA
  Oral presentation: C.V. Stevens, E. Delbeke, S. Mincke, K. Van Geem, *"Wash and go" approach: Chemical Modification of Biopolymers for Green Surfactants*
- 2nd International Symposium and Workshop of the Global Green Chemistry Centres (G2C2), 24-26/08/2014, Cape Town, South Africa
  Oral presentation: C.V. Stevens, E. Delbeke, S. Mincke, A. Verlee, *Chemical Modification of Renewables for Sustainable Chemicals Development*
- 248th American Chemical Society National Meeting & Exposition, 10-14/08/2014, San Francisco, USA
  Oral presentation: E.I.P. Delbeke, J. Van den Abeele, K.M. Van Geem, C.V. Stevens, *Innovative Sophorolipid Analogues as Green Surface-Active Agents*

- 14th Belgian Organic Synthesis Symposium (BOSS XIV), 13-18/07/2014, Louvain-la-Neuve, Belgium

  Poster presentation: E.I.P. Delbeke, J. Van den Abeele, B.I. Roman, K.M. Van Geem, C.V. Stevens, *Green Surface-Active Compounds from the Chemical Modification of Sophorolipids*

  Poster presentation: I. Wauters, E. Delbeke, T.S.A. Heugebaert, B. Roman, K. Van Hecke, C.V. Stevens, *Cyclization of cyclo(Trp,Pro) Toward 2,6-bridged piperazine-3-ones*
- 10th International Conference on Renewable Resources and Biorefineries (RRB10), 4-6/06/2014, Valladolid, Spain

  Poster presentation: E.I.P. Delbeke, J. Van den Abeele, K.M. Van Geem, C.V. Stevens, *Synthesis of innovative sophorolipid analogues*

  Poster presentation: E. Uitterhaegen, K.A. Sampaio, E.I.P. Delbeke, Q.H. Nguyen, P. Evon, O. Merah, C.V. Stevens, T. Talou, *Coriander Biorefinery – Reactive Extrusion and Oleochemistry*
- 2014 Belgian Peptide Group Meeting, 10-11/02/2014, Ghent, Belgium

  Poster presentation: I. Wauters, E. Delbeke, T.S.A. Heugebaert, B. Roman, K. Van Hecke, C.V. Stevens, *Cyclization of cyclo(Trp,Pro) Toward 2,6-bridged piperazine-3-ones*

Skills

General laboratory skills
Automated flash chromatography responsible
LC-MS analysis responsible
Ozonolysis equipment
Supervision of Master and internship students
Supervision of practical laboratory sessions

8. References

1. Brown, R. C. *Biorenewable resources. Engineering new products from agriculture.*; Blackwell Publishing: USA, 2003.
2. Metzger, J. O.; Eissen, M. *C. R. Chim.* **2004**, *7*, 569-581.
3. Koutinas, A. A.; Du, C.; Wang, R. H.; Webb, C. Production of Chemicals from Biomass. In *Introduction to chemicals from biomass*; 1 ed.; Clark, J. H.; Deswarte, F. E. I., Eds.; John Wiley & Sons: UK, 2008, pp 77-101.
4. Vandermeulen, V.; Van der Steen, M.; Stevens, C. V.; Van Huylenbroeck, G. *Biofuel. Bioprod. Biorefin.* **2012**, *6*, 453-466.
5. Clark, J. H.; Budarin, V.; Deswarte, F. E. I.; Hardy, J. J. E.; Kerton, F. M.; Hunt, A. J.; Luque, R.; Macquarrie, D. J.; Milkowski, K.; Rodriguez, A.; Samuel, O.; Tavener, S. J.; White, R. J.; Wilson, A. J. *Green Chem.* **2006**, *8*, 853-860.
6. Menrad, K.; Klein, A.; Kurka, S. *Biofuel. Bioprod. Biorefin.* **2009**, *3*, 384-394.
7. Cherubini, F.; Jungmeier, G.; Wellisch, M.; Willke, T.; Skiadas, I.; Van Ree, R.; de Jong, E. *Biofuel. Bioprod. Biorefin.* **2009**, *3*, 534-546.
8. Budarin, V. L.; Shuttleworth, P. S.; Dodson, J. R.; Hunt, A. J.; Lanigan, B.; Marriott, R.; Milkowski, K. J.; Wilson, A. J.; Breeden, S. W.; Fan, J. J.; Sin, E. H. K.; Clark, J. H. *Energy Environ. Sci.* **2011**, *4*, 471-479.
9. Chambers, M.; Muecke, M. *J. Green Build.* **2010**, *5*, 91-107.
10. Van Bogaert, I. N. A.; Saerens, K.; De Muynck, C.; Develter, D.; Soetaert, W.; Vandamme, E. J. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 23-34.
11. Van Bogaert, I. N. A.; Zhang, J. X.; Soetaert, W. *Process Biochem.* **2011**, *46*, 821-833.
12. Baviere, M.; Degouy, D.; Lecourtier, J. Process for washing solid particles comprising a sophoroside solution. US Pat. 5 326 407, 1993.
13. Pesce, L. A biotechnological method for the regeneration of hydrocarbons from dregs and muds, on the base of biosurfactants. WO 2002062495, 2001.
14. Ducreux, J.; Ballerini, D.; Baviere, M.; Bocard, C.; Monin, N. Composition containing a surface active compound and glycolipids and decontamination process for a porous medium polluted by hydrocarbons. US Pat. 5 654 192, 1995.
15. Mulligan, C. N.; Yong, R. N.; Gibbs, B. F. *J. Hazard. Mater.* **2001**, *85*, 111-125.
16. Mulligan, C. N.; Yong, R. N.; Gibbs, B. F. *Eng. Geol.* **2001**, *60*, 371-380.
17. Schippers, C.; Gessner, K.; Muller, T.; Scheper, T. *J. Biotechnol.* **2000**, *83*, 189-198.
18. Morya, V. K.; Ahn, C.; Jeon, S.; Kim, E. K. *Mini-Rev. Med. Chem.* **2013**, *13*, 1761-1768.
19. Delbeke, E. I. P.; Movsisyan, M.; Van Geem, K. M.; Stevens, C. V. *Green Chem.* **2016**, *18*, 76-104.
20. Baccile, N.; Nassif, N.; Malfatti, L.; Van Bogaert, I. N. A.; Soetaert, W.; Pehau-Arnaudet, G.; Babonneau, F. *Green Chem.* **2010**, *12*, 1564-1567.
21. Baccile, N.; Babonneau, F.; Jestin, J.; Pehau-Arnaudet, G.; Van Bogaert, I. *ACS Nano* **2012**, *6*, 4763-4776.
22. Roelants, S. L. K. W.; Ciesielska, K.; De Maeseneire, S. L.; Everaert, B.; Denon, Q.; Moens, H.; Vanlerberghe, B.; Van Bogaert, I. N. A.; Van der Meeren, P.; De Vreese, B.; Soetaert, W. *Biotechnol. Bioeng.* **2016**, *113*, 550-559.
23. Delbeke, E. I. P.; Roelants, S. L. K. W.; Matthijs, N.; Everaert, B.; Soetaert, W.; Coenye, T.; Van Geem, K. M.; Stevens, C. V. *Ind. Eng. Chem. Res.* **2016**, accepted.
24. Kjellin, M.; Johansson, I., *Surfactants from renewable resources*. John Wiley & Sons, Ltd: UK, 2010, p 320.
25. Sajna, K. V.; Höfer, R.; Sukumaran, R. K.; Gottumukkala, L. D.; Pandey, A. White Biotechnology in Biosurfactants. In *Industrial Biorefineries and White Biotechnology*; Pandey, A.; Höfer, R.; Taherzadeh, M.; Nampoothiri, K. M.; Larroche, C., Eds.; Elsevier: Amsterdam, Oxford, Waltham, 2015, pp 499-521.

26. Van Bogaert, I.; Fleurackers, S.; Van Kerrebroeck, S.; Develter, D.; Soetaert, W. *Biotechnol. Bioeng.* **2011**, *108*, 734-741.
27. Brakemeier, A.; Wullbrandt, D.; Lang, S. *Appl. Microbiol. Biotechnol.* **1998**, *50*, 161-166.
28. Develter, D.; Fleurackers, S. A method for the production of short chained glycolipids. EP 1 953 237 A1, 2008.
29. Peng, Y.; Totsingan, F.; Meier, M. A. R.; Steinmann, M.; Wurm, F.; Koh, A.; Gross, R. A. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 217-228.
30. Asmer, H. J.; Lang, S.; Wagner, F.; Wray, V. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1460-1466.
31. Ciesielska, K.; Van Bogaert, I. N.; Chevineau, S.; Li, B.; Groeneboer, S.; Soetaert, W.; Van de Peer, Y.; Devreese, B. *J. Proteomics* **2014**, *98*, 159-174.
32. Hu, Y. M.; Ju, L. K. *J. Biotechnol.* **2001**, *87*, 263-272.
33. Baccile, N.; Cuvier, A. S.; Valotteau, C.; Van Bogaert, I. N. A. *Eur. J. Lipid Sci. Technol.* **2013**, *115*, 1404-1412.
34. Hirata, Y.; Ryu, M.; Oda, Y.; Igarashi, K.; Nagatsuka, A.; Furuta, T.; Sugiura, M. *J. Biosci. Bioeng.* **2009**, *108*, 142-146.
35. Develter, D. W. G.; Lauryssen, L. M. L. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 628-638.
36. Ma, X. J.; Li, H.; Song, X. *J. Colloid Interface Sci.* **2012**, *376*, 165-172.
37. Hillion, G.; Marchal, R.; Stoltz, C.; Borzeix, F. Use of a sophorolipid to provide free radical formation inhibiting activity or elastase inhibiting activity. US Pat. 5 756 471, 1995.
38. Borzeix Concaix, F. Use of sophorolipids comprising diacetyl lactones as agent for stimulating skin fibroblast metabolism. US Pat. 6 596 265, 1999.
39. Mager, H.; Röthlisberger, R.; Wagner, F. Use of sophorolipid-lactone for the treatment of dandruffs and body odour. EP 0 209 783, 1986.
40. Maingault, M. Utilization of sophorolipids as therapeutically active substances or cosmetic products, in particular for the treatment of the skin. US Pat. 5 981 497, 1996.
41. Andre, P.; Pellicier, F. Cosmetic use of sophorolipids as subcutaneous adipose cushion regulating agents and slimming application. WO 2004108063, 2004.
42. Ito, S.; Kinta, M.; Inoue, S. *Agric. Biol. Chem.* **1980**, *44*, 2221-2223.
43. Gross, R. A.; Shah, V. Method for neutralizing fungi using sophorolipids and antifungal sophorolipids for use therein. US Pat. 8 796 228, 2009.
44. Kulakovskaya, E.; Baskunov, B.; Zvonarev, A. *J. Oleo Sci.* **2014**, *63*, 701-707.
45. Shah, V.; Badia, D.; Ratsep, P. *Antimicrob. Agents Chemother.* **2007**, *51*, 397-400.
46. Gross, R. A.; Shah, V. Antimicrobial properties of various forms of sophorolipids. WO 2004044216, 2002.
47. Sleiman, J. N.; Kohlhoff, S. A.; Roblin, P. M.; Wallner, S.; Gross, R.; Hammerschlag, M. R.; Zenilman, M. E.; Bluth, M. H. *Ann. Clin. Lab. Sci.* **2009**, *39*, 60-63.
48. Pulate, V. D.; Bhagwat, S.; Prabhune, A. *J. Surfactants Deterg.* **2013**, *16*, 173-181.
49. Diaz De Rienzo, M. A.; Banat, I. M.; Dolman, B.; Winterburn, J.; Martin, P. J. *New Biotechnol.* **2015**, DOI 10.1016/j.nbt.2015.02.009.
50. Joshi-Navare, K.; Prabhune, A. *BioMed Res. Int.* **2013**, DOI 10.1155/2013/512495.
51. Sun, X. X.; Choi, J. K.; Kim, E. K. *J. Exp. Mar. Biol. Ecol.* **2004**, *304*, 35-49.
52. Sun, X.; Kim, E.; Sun, S. *Chin. J. Oceanol. Limnol.* **2010**, *28*, 1240-1247.
53. Sun, X. X.; Lee, Y. J.; Choi, J. K.; Kim, E. K. *Mar. Pollut. Bull.* **2004**, *48*, 863-872.
54. Isoda, H.; Kitamoto, D.; Shinmoto, H.; Matsumura, M.; Nakahara, T. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 609-614.
55. Chen, J.; Song, X.; Zhang, H.; Qu, Y. *Enzyme Microb. Technol.* **2006**, *39*, 501-506.
56. Chen, J.; Song, X.; Zhang, H.; Qu, Y. B.; Miao, J. Y. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 52-59.
57. Fu, S. L.; Wallner, S. R.; Bowne, W. B.; Hagler, M. D.; Zenilman, M. E.; Gross, R.; Bluth, M. H. *J. Surg. Res.* **2008**, *148*, 77-82.
58. Shao, L. J.; Song, X.; Ma, X. J.; Li, H.; Qu, Y. B. *J. Surg. Res.* **2012**, *173*, 286-291.
59. Ribeiro, I. A. C.; Faustino, C. M. C.; Guerreiro, P. S.; Frade, R. F. M.; Bronze, M. R.; Castro, M. F.; Ribeiro, M. H. L. *J. Mol. Recognit.* **2015**, *28*, 155-165.

60. Joshi-Navare, K.; Shiras, A.; Prabhune, A. *Biotechnol. J.* **2011**, *6*, 509-512.
61. Bluth, M. H.; Kandil, E.; Mueller, C. M.; Shah, V.; Lin, Y. Y.; Zhang, H.; Dresner, L.; Lempert, L.; Nowakowski, M.; Gross, R.; Schulze, R.; Zenilman, M. E. *Crit. Care Med.* **2006**, *34*, 188-195.
62. Fu, S. L.; Mueller, C.; Lin, Y. Y.; Viterbo, D.; Pierre, J.; Shah, V.; Gross, R.; Schulze, R.; Zenilman, M. *J. Am. Coll. Surgeons* **2007**, *205*, S44-S44.
63. Hagler, M.; Smith-Norowitz, T. A.; Chicel, S.; Wallner, S. R.; Viterbo, D.; Mueller, C. M.; Gross, R.; Nowakowski, M.; Schulze, R.; Zenilman, M. E.; Bluth, M. H. *J. Allergy Clin. Immunol.* **2007**, *119*, S263-S263.
64. Hardin, R.; Pierre, J.; Schulze, R.; Mueller, C. M.; Fu, S. L.; Wallner, S. R.; Stanek, A.; Shah, V.; Gross, R. A.; Weedon, J.; Nowakowski, M.; Zenilman, M. E.; Bluth, M. H. *J. Surg. Res.* **2007**, *142*, 314-319.
65. Bluth, M. H.; Fu, S. L.; Fu, A.; Stanek, A.; Smith-Norowitz, T. A.; Wallner, S. R.; Gross, R. A.; Nowakowski, M.; Zenilman, M. E. *J. Allergy Clin. Immunol.* **2008**, *121*, S2-S2.
66. Gross, R. A.; Shah, V.; Doncel, G. F. Spermicidal and virucidal properties of various forms of sophorolipids. US Pat. 2011/0 223 239, 2011.
67. Shah, V.; Doncel, G. F.; Seyoum, T.; Eaton, K. M.; Zalenskaya, I.; Hagver, R.; Azim, A.; Gross, R. *Antimicrob. Agents Chemother.* **2005**, *49*, 4093-4100.
68. Gross, R. A.; Shah, V. Anti-herpes virus properties of various forms of sophorolipids. WO 2007130738, 2007.
69. Gorin, P. A.; Spencer, J. F. T.; Tulloch, A. P. *Can. J. Chem.* **1961**, *39*, 846-855.
70. Tulloch, A. P.; Spencer, J. F. T.; Deinema, M. H. *Can. J. Chem.* **1968**, *46*, 345-348.
71. Tulloch, A. P.; Hill, A.; Spencer, J. F. T. *Can. J. Chem.* **1968**, *46*, 3337-3351.
72. Tulloch, A. P.; Spencer, J. F. T. *J. Org. Chem.* **1972**, *37*, 2868-2870.
73. Furstner, A.; Radkowski, K.; Grabowski, J.; Wirtz, C.; Mynott, R. *J. Org. Chem.* **2000**, *65*, 8758-8762.
74. Bisht, K. S.; Gross, R. A.; Kaplan, D. L. *J. Org. Chem.* **1999**, *64*, 780-789.
75. Peng, Y. F.; Munoz-Pinto, D. J.; Chen, M. T.; Decatur, J.; Hahn, M.; Gross, R. A. *Biomacromolecules* **2014**, *15*, 4214-4227.
76. Singh, S. K.; Felse, A. P.; Nunez, A.; Foglia, T. A.; Gross, R. A. *J. Org. Chem.* **2003**, *68*, 5466-5477.
77. Recke, V. K.; Gerlitzki, M.; Hausmann, R.; Syldatk, C.; Wray, V.; Tokuda, H.; Suzuki, N.; Lang, S. *Eur. J. Lipid Sci. Technol.* **2013**, *115*, 452-463.
78. Rau, U.; Heckmann, R.; Wray, V.; Lang, S. *Biotechnol. Lett.* **1999**, *21*, 973-977.
79. Rau, U.; Hammen, S.; Heckmann, R.; Wray, V.; Lang, S. *Ind. Crops Prod.* **2001**, *13*, 85-92.
80. Imura, T.; Masuda, Y.; Minamikawa, H.; Fukuoka, T.; Konishi, M.; Morita, T.; Sakai, H.; Abe, M.; Kitamoto, D. *J. Oleo Sci.* **2010**, *59*, 495-501.
81. Zerkowski, J. A.; Solaiman, D. K. Y.; Ashby, R. D.; Foglia, T. A. *J. Surfactants Deterg.* **2006**, *9*, 57-62.
82. Inoue, S.; Kimura, I.; Kinta, M. Hydroxyalkyl-etherified glycolipid ester. US Pat. 4 195 177, 1978.
83. Kawano, J.; Utsugi, T.; Inoue, S.; Hayashi, S. Powdered compressed cosmetic material. US Pat. 4 305 931, 1979.
84. Kawano, J.; Suzuki, T.; Inoue, S.; Hayashi, S. Stick-shaped cosmetic material. US Pat. 4 305 929, 1979.
85. Tsutsumi, H.; Kawano, J.; Inoue, S.; Hayashi, S. Cosmetic composition. US Pat. 4 305 961, 1979.
86. Zhang, L.; Somasundaran, P.; Singh, S. K.; Felse, A. P.; Gross, R. *Colloids Surf., A* **2004**, *240*, 75-82.
87. Gupta, R.; Prabhune, A. A. *Biotechnol. Lett.* **2012**, *34*, 701-707.
88. Gupta, R.; Uma, S. K.; Prabhune, A. *Res. J. Biotechnol.* **2012**, *7*, 40-45.
89. Carr, J. A.; Bisht, K. S. *Tetrahedron* **2003**, *59*, 7713-7724.
90. Nunez, A.; Foglia, T. A.; Ashby, R. *Biotechnol. Lett.* **2003**, *25*, 1291-1297.
91. Azim, A.; Shah, V.; Doncel, G. F.; Peterson, N.; Gao, W.; Gross, R. *Bioconjugate Chem.* **2006**, *17*, 1523-1529.
92. Schofield, M. H.; Thavasi, T. R.; Gross, R. A. Modified sophorolipids for the inhibition of plant pathogens. US Pat. 2013/0 085 067, 2012.

93. Bisht, K. S.; Gao, W.; Gross, R. A. *Macromolecules* **2000**, *33*, 6208-6210.
94. Gao, W.; Hagver, R.; Shah, V.; Xie, W. C.; Gross, R. A.; Ilker, M. F.; Bell, C.; Burke, K. A.; Coughlin, E. B. *Macromolecules* **2007**, *40*, 145-147.
95. Zini, E.; Gazzano, M.; Scandola, M.; Wallner, S. R.; Gross, R. A. *Macromolecules* **2008**, *41*, 7463-7468.
96. Peng, Y. F.; Decatur, J.; Meier, M. A. R.; Gross, R. A. *Macromolecules* **2013**, *46*, 3293-3300.
97. Hu, Y.; Ju, L.-K. *Biotechnol. Prog.* **2003**, *19*, 303-311.
98. Develter, D.; Fleurackers, S. A method for the production of short chained glycolipids. EP 1 953 237, 2007.
99. Peng, Y.; Totsingan, F.; Meier, M. A. R.; Steinmann, M.; Wurm, F.; Koh, A.; Gross, R. A. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 217-228.
100. Zerkowski, J. A.; Solaiman, D. K. Y. *J. Am. Oil Chem. Soc.* **2006**, *83*, 621-628.
101. Zerkowski, J. A.; Solaiman, D. K. Y. *J. Am. Oil Chem. Soc.* **2007**, *84*, 463-471.
102. Hoffmann, N.; Pietruszka, J.; Soffing, C. *Adv. Synth. Catal.* **2012**, *354*, 959-963.
103. Kasture, M.; Singh, S.; Patel, P.; Joy, P. A.; Prabhune, A. A.; Ramana, C. V.; Prasad, B. L. V. *Langmuir* **2007**, *23*, 11409-11412.
104. Kasture, M. B.; Patel, P.; Prabhune, A. A.; Ramana, C. V.; Kulkarni, A. A.; Prasad, B. L. V. *J. Chem.Sci.* **2008**, *120*, 515-520.
105. Singh, S.; Patel, P.; Jaiswal, S.; Prabhune, A. A.; Ramana, C. V.; Prasad, B. L. V. *New J. Chem.* **2009**, *33*, 646-652.
106. Kumar, D. V. R.; Kasture, M.; Prabhune, A. A.; Ramana, C. V.; Prasad, B. L. V.; Kulkarni, A. A. *Green Chem.* **2010**, *12*, 609-615.
107. Kumar, D. V. R.; Prasad, B. L. V.; Kulkarni, A. A. *Chem. Eng. J.* **2012**, *192*, 357-368.
108. D'Britto, V.; Kapse, H.; Babrekar, H.; Prabhune, A. A.; Bhoraskar, S. V.; Premnath, V.; Prasad, B. L. V. *Nanoscale* **2011**, *3*, 2957-2963.
109. Dhar, S.; Reddy, E. M.; Prabhune, A.; Pokharkar, V.; Shiras, A.; Prasad, B. L. V. *Nanoscale* **2011**, *3*, 575-580.
110. Baccile, N.; Noiville, R.; Stievano, L.; Van Bogaert, I. *Phys. Chem. Chem. Phys.* **2013**, *15*, 1606-1620.
111. Singh, P. K.; Mukherji, R.; Joshi-Navare, K.; Banerjee, A.; Gokhale, R.; Nagane, S.; Prabhune, A.; Ogale, S. *Green Chem.* **2013**, *15*, 943-953.
112. Singh, P.; Joshi, K.; Guin, D.; Prabhune, A. A. *RSC Adv.* **2013**, *3*, 22319-22325.
113. Basak, G.; Das, D.; Das, N. *J. Microbiol. Biotechnol.* **2014**, *24*, 87-96.
114. Schwartz, C.; Raible, J.; Mott, K.; Dussault, P. H. *Tetrahedron* **2006**, *62*, 10747-10752.
115. Schiaffo, C. E. An improved procedure for the alkene ozonolysis - Exploring a new structural paradigm for peroxide antimalarials. The Graduate College, University of Nebraska, Lincoln, Nebraska, 2011.
116. Kyasa, S.; Fisher, T. J.; Dussault, P. H. *Synthesis-Stuttgart* **2011**, 3475-3481.
117. Roe, R. M.; Kallapur, V.; Linderman, R. J.; Viviani, F. *Pestic. Biochem. Physiol.* **2005**, *83*, 140-154.
118. Tobiszewski, M.; Marc, M.; Galuszka, A.; Namiesnik, J. *Molecules* **2015**, *20*, 10928-10946.
119. Cahoon, E. B.; Dormann, P.; Ohlrogge, J. B. *Prog. Lipid Res.* **1994**, *33*, 155-163.
120. Placek, L. L. *J. Am. Oil Chem. Soc.* **1963**, *40*, 319-329.
121. Uitterhaegen, E.; Nguyen, Q. H.; Sampaio, K. A.; Stevens, C. V.; Merah, O.; Talou, T.; Rigal, L.; Evon, P. *J. Am. Oil Chem. Soc.* **2015**, *92*, 1219-1233.
122. Barrett, K. E.; Green, M. R.; Hu, H.-L.; Parmar, P.; Powell, J. R.; Rawlings, A. V. Skin care composition. US Pat. 6 455 057, 2000.
123. Alaluf, S.; Hu, H.-L.; Green, M. R.; Powell, J. R.; Rawlings, A. V.; Rogers, J. S.; Watkinson, A.; Cain, F. W. Cosmetic use of petroselinic acid. EP 1 013 178, 1999.
124. Weinkauf, R.; Santhanam, U.; Palanker, L. R.; Januario, T. G.; Brinker, A. Petroselinic acid as an anti-irritant in compositions containing alpha hydroxy acids. US Pat. 6 022 896, 1998.
125. Dierker, M.; Schafer, H. J. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 122-136.
126. Heimerma.Wh; Gordon, D. T.; Kowalysh.De; Jensen, R. G.; Holman, R. T. *Lipids* **1973**, *8*, 45-46.

127. Weber, N.; Richter, K. D.; Schulte, E.; Mukherjee, K. D. *J. Nutr.* **1995**, *125*, 1563-1568.
128. Shukla, S.; Gupta, S. Coriander. In *Molecular targets and therapeutic uses of spices: Modern uses for ancient medicine*; Aggarwal, B. B.; Kunnumakkara, A. B., Eds.; World Scientific: Singapore, 2009, pp 149-171.
129. Lang, S.; Brakemeier, A.; Heckmann, R.; Spockner, S.; Rau, U. *Chim. Oggi* **2000**, *18*, 76-79.
130. Jiang, Y. J.; Geng, T.; Li, Q. X. *J. Surfactants Deterg.* **2012**, *15*, 67-71.
131. Gentamicin(e) product monograph. Corporation, B. 2012, p 39. Retrieved 20/02/2016.
132. WHO Model List of Essential Medicines. Organization, W. H. 2013, p 43. Retrieved 20/02/2016.
133. Ioannou, C. J.; Hanlon, G. W.; Denyer, S. P. *Antimicrob. Agents Chemother.* **2007**, *51*, 296-306.
134. Hegstad, K.; Langsrud, S.; Lunestad, B. T.; Scheie, A. A.; Sunde, M.; Yazdankhah, S. P. *Microb. Drug. Resist.* **2010**, *16*, 91-104.
135. Meier, L.; Monteiro, G. C.; Baldissera, R. A. M.; Sa, M. M. *J. Braz. Chem. Soc.* **2010**, *21*, 859-866.
136. Perche, F.; Benvegnu, T.; Berchel, M.; Lebegue, L.; Pichon, C.; Jaffres, P. A.; Midoux, P. *Nanomedicine* **2011**, *7*, 445-453.
137. Leung, A. K. K.; Tam, Y. Y. C.; Cullis, P. R. *Adv. Genet.* **2014**, *88*, 71-110.
138. Guo, X.; Huang, L. *Acc. Chem. Res.* **2012**, *45*, 971-979.
139. Berchel, M.; Le Gall, T.; Couthon-Gourves, H.; Haelters, J. P.; Montier, T.; Midoux, P.; Lehn, P.; Jaffres, P. A. *Biochimie* **2012**, *94*, 33-41.
140. Montier, T.; Benvegnu, T.; Jaffres, P. A.; Yaouanc, J. J.; Lehn, P. *Curr. Gene Ther.* **2008**, *8*, 296-312.
141. Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 7413-7417.
142. Billiet, L.; Gomez, J. P.; Berchel, M.; Jaffres, P. A.; Le Gall, T.; Montier, T.; Bertrand, E.; Cheradame, H.; Guegan, P.; Mevel, M.; Pitard, B.; Benvegnu, T.; Lehn, P.; Pichon, C.; Midoux, P. *Biomaterials* **2012**, *33*, 2980-2990.
143. Laurent, V.; Fraix, A.; Montier, T.; Cammas-Marion, S.; Ribault, C.; Benvegnu, T.; Jaffres, P. A.; Loyer, P. *Biotechnol. J.* **2010**, *5*, 314-320.
144. Nantz, M. H.; Dicus, C. W.; Hilliard, B.; Yellayi, S.; Zou, S. M.; Hecker, J. G. *Mol. Pharmaceutics* **2010**, *7*, 786-794.
145. Alton, E. W. F. W.; Armstrong, D. K.; Ashby, D.; Bayfield, K. J.; Bilton, D.; Bloomfield, E. V.; Boyd, A. C.; Brand, J.; Buchan, R.; Calcedo, R.; Carvelli, P.; Chan, M.; Cheng, S. H.; Collie, D. D. S.; Cunningham, S.; Davidson, H. E.; Davies, G.; Davies, J. C.; Davies, L. A.; Dewar, M. H.; Doherty, A.; Donovan, J.; Dwyer, N. S.; Elgmati, H. I.; Featherstone, R. F.; Gavino, J.; Gea-Sorli, S.; Geddes, D. M.; Gibson, J. S. R.; Gill, D. R.; Greening, A. P.; Griesenbach, U.; Hansell, D. M.; Harman, K.; Higgins, T. E.; Hodges, S. L.; Hyde, S. C.; Hyndman, L.; Innes, J. A.; Jacob, J.; Jones, N.; Keogh, B. F.; Limberis, M. P.; Lloyd-Evans, P.; Maclean, A. W.; Manvell, M. C.; McCormick, D.; McGovern, M.; McLachlan, G.; Meng, C. X.; Montero, M. A.; Milligan, H.; Moyce, L. J.; Murray, G. D.; Nicholson, A. G.; Osadolor, T.; Parra-Leiton, J.; Porteous, D. J.; Pringle, I. A.; Punch, E. K.; Pytel, K. M.; Quittner, A. L.; Rivellini, G.; Saunders, C. J.; Scheule, R. K.; Sheard, S.; Simmonds, N. J.; Smith, K.; Smith, S. N.; Soussi, N.; Soussi, S.; Spearing, E. J.; Stevenson, B. J.; Sumner-Jones, S. G.; Turkkila, M.; Ureta, R. P.; Waller, M. D.; Wasowicz, M. Y.; Wilson, J. M.; Wolstenholme-Hogg, P.; Co, U. C. F. G. T. *Lancet Respir. Med.* **2015**, *3*, 684-691.
146. Lindberg, M. F.; Le Gall, T.; Carmoy, N.; Berchel, M.; Hyde, S. C.; Gill, D. R.; Jaffres, P. A.; Lehn, P.; Montier, T. *Biomaterials* **2015**, *59*, 1-11.
147. Sun, J. Y.; Anand-Jawa, V.; Chatterjee, S.; Wong, K. K. *Gene Ther.* **2003**, *10*, 964-976.
148. Midoux, P.; Pichon, C.; Yaouanc, J. J.; Jaffres, P. A. *Br. J. Pharmacol.* **2009**, *157*, 166-178.
149. Liu, D. L.; Qiao, W. H.; Li, Z. S.; Chen, Y. X.; Cui, X. Y.; Li, K.; Yu, L. H.; Yan, K. L.; Zhu, L. M.; Guo, Y. L.; Cheng, L. B. *Chem. Biol. Drug Des.* **2008**, *71*, 336-344.
150. Le Corre, S. S.; Berchel, M.; Le Gall, T.; Haelters, J. P.; Lehn, P.; Montier, T.; Jaffres, P. A. *Eur. J. Org. Chem.* **2014**, 8041-8048.
151. Le Gall, T.; Loizeau, D.; Picquet, E.; Carmoy, N.; Yaouanc, J. J.; Burel-Deschamps, L.; Delepine, P.; Giamarchi, P.; Jaffres, P. A.; Lehn, P.; Montier, T. *J. Med. Chem.* **2010**, *53*, 1496-1508.

152. Lindberg, M. F.; Carmoy, N.; Le Gall, T.; Fraix, A.; Berchel, M.; Lorilleux, C.; Couthon-Gourves, H.; Bellaud, P.; Fautrel, A.; Jaffres, P. A.; Lehn, P.; Montier, T. *Biomaterials* **2012**, *33*, 6240-6253.
153. Koynova, R.; Tenchov, B.; Wang, L.; MacDonald, R. C. *Mol. Pharmaceutics* **2009**, *6*, 951-958.
154. Rosenzweig, H. S.; Rakhmanova, V. A.; McIntosh, T. J.; MacDonald, R. C. *Bioconjugate Chem.* **2000**, *11*, 306-313.
155. MacDonald, R. C.; Rakhmanova, V. A.; Choi, K. L.; Rosenzweig, H. S.; Lahiri, M. K. *J. Pharm. Sci.* **1999**, *88*, 896-904.
156. Belmont, P.; Aissaoui, A.; Hauchecorne, M.; Oudrhiri, N.; Petit, L.; Vigneron, J. P.; Lehn, J. M.; Lehn, P. *J. Gene Med.* **2002**, *4*, 517-526.
157. Sainlos, M.; Belmont, P.; Vigneron, J. P.; Lehn, P.; Lehn, J. M. *Eur. J. Org. Chem.* **2003**, 2764-2774.
158. Niyomtham, N.; Apiratikul, N.; Suksen, K.; Opanasopit, P.; Yingyongnarongkul, B. E. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 496-503.
159. Lamarche, F.; Mevel, M.; Montier, T.; Burel-Deschamps, L.; Giamarchi, P.; Tripier, R.; Delepine, P.; Le Gall, T.; Cartier, D.; Lehn, P.; Jaffrees, P. A.; Clement, J. C. *Bioconjugate Chem.* **2007**, *18*, 1575-1582.
160. Paris, C.; Moreau, V.; Deglane, G.; Karim, L.; Couturier, B.; Bonnet, M. E.; Kedinger, V.; Messmer, M.; Bolcato-Bellemin, A. L.; Behr, J. P.; Erbacher, P.; Lenne-Samuel, N. *Mol. Pharmaceutics* **2012**, *9*, 3464-3475.
161. Paecharoenchai, O.; Niyomtham, N.; Ngawhirunpat, T.; Rojanarata, T.; Yingyongnarongkul, B. E.; Opanasopit, P. *J. Drug Targeting* **2012**, *20*, 783-792.
162. Fraix, A.; Montier, T.; Le Gall, T.; Sevrain, C. M.; Carmoy, N.; Lindberg, M. F.; Lehn, P.; Jaffres, P. A. *Org. Biomol. Chem.* **2012**, *10*, 2051-2058.
163. Fukuda, S.; Terasawa, M.; Shiomi, K. *Food Chem. Toxicol.* **2011**, *49*, 1598-1603.
164. Le Gall, T.; Berchel, M.; Le Hir, S.; Fraix, A.; Salaun, J. Y.; Ferec, C.; Lehn, P.; Jaffres, P. A.; Montier, T. *Adv. Healthcare Mater.* **2013**, *2*, 1513-1524.
165. Sharma, V. D.; Lees, J.; Hoffman, N. E.; Brailoiu, E.; Madesh, M.; Wunder, S. L.; Ilies, M. A. *Mol. Pharmaceutics* **2014**, *11*, 545-559.
166. Han, K. H. *Nucleic Acids Res.* **1996**, *24*, 4362-4363.
167. Pinnaduwage, P.; Schmitt, L.; Huang, L. *Biochim. Biophys. Acta* **1989**, *985*, 33-37.
168. Berchel, M.; Le Gall, T.; Haelters, J. P.; Lehn, P.; Montier, T.; Jaffres, P. A. *Mol. Pharmaceutics* **2015**, *12*, 1902-1910.
169. Le Corre, S. S.; Belmadi, N.; Berchel, M.; Le Gall, T.; Haelters, J. P.; Lehn, P.; Montier, T.; Jaffres, P. A. *Org. Biomol. Chem.* **2015**, *13*, 1122-1132.
170. Oldenhove de Guertechin, L. Surfactants: Classification. In *Handbook of cosmetic science and and technology*; 3th ed.; Barel, A. O.; Paye, M.; Maibach, H. I., Eds.; Informa Healthcare USA, Inc.: New York, 2009, pp 769-786.
171. Santora, V. J.; Covel, J. A.; Hayashi, R.; Webb, R. R.; Ren, A. S.; Chen, W. G.; Duffield, J. J.; Ibarra, J. B.; Pulley, M.; Semple, G.; Weinhouse, M. I. Modulators of the h3 receptor useful for the treatment of disorders related thereto. WO 2007061741, 2007.
172. Guarna, A.; Occhiato, E. G.; Pizzetti, M.; Scarpi, D.; Sisi, S.; van Sterkenburg, M. *Tetrahedron: Asymmetry* **2000**, *11*, 4227-4238.
173. Fuhrhop, A. H.; Wang, T. Y. *Chem. Rev.* **2004**, *104*, 2901-2937.
174. Benvegnu, T.; Rethore, G.; Brard, M.; Richter, W.; Plusquellec, D. *Chem. Commun.* **2005**, 5536-5538.
175. Cornell, B. A.; BraachMaksvytis, V. L. B.; King, L. G.; Osman, P. D. J.; Raguse, B.; Wieczorek, L.; Pace, R. J. *Nature* **1997**, *387*, 580-583.
176. Nuraje, N.; Bai, H. Y.; Su, K. *Prog. Polym. Sci.* **2013**, *38*, 302-343.
177. De Vos, N. Development of new ionic liquids based on a 7-aza- or 7-thiabicyclo[2.2.1] heptane skeleton. Department of Sustainable Organic Chemistry and Technology, Ghent University, Ghent, 2013.
178. Otte, K. B.; Kirtz, M.; Nestl, B. M.; Hauer, B. *ChemSusChem* **2013**, *6*, 2149-2156.

179. Roberge, D. M.; Ducry, L.; Bieler, N.; Cretton, P.; Zimmermann, B. *Chem. Eng. Technol.* **2005**, *28*, 318-323.
180. Van den Driessche, F.; Rigole, P.; Brackman, G.; Coenye, T. *J. Microbiol. Methods* **2014**, *98*, 31-34.
181. Fraix, A.; Le Gall, T.; Berchel, M.; Denis, C.; Lehn, P.; Montier, T.; Jaffres, P. A. *Org. Biomol. Chem.* **2013**, *11*, 1650-1658.